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(54) Title: MAGNETIC-RESPONSIVE DENDRIMER COMPOSITION AND ITS USE IN MAGNETIC RESONANCE ANALYSIS

(57) Abstract: A magnetic probe that includes magnetic-responsive metal (oxyhydr)oxide particles stabilized by a dendrimer. The magnetic probe may be a magnetic resonance (MR) contrast agent in the form of an aqueous suspension that includes iron oxide particles synthesized in the presence of a carboxy-terminated poly(amidoamine) dendrimer. Such magnetic probes are useful for labeling living cells to render the cells magnetic resonance sensitive by contacting the magnetic probe with the cells to be labeled and allowing the metal (oxyhydr)oxide-dendrimer composition to be internalized by the cell. The magnetic probes can be made by *in situ* oxidizing a metal ion-containing material in the presence of a dendrimer.

MAGNETIC-RESPONSIVE DENDRIMER COMPOSITION AND ITS USE IN MAGNETIC RESONANCE ANALYSIS

This application claims priority of U.S. Provisional Patent Application No. 60/193,360 filed March 31, 2000, incorporated herein by reference. Co-pending, commonly-assigned PCT Application No. US00/13826 "Magnetic Resonance Tracking of Magnetically Labeled Cells" filed May 19, 2000 (published November 30, 2000) discloses related subject matter.

Field of the Invention

The present invention relates to an organometal material useful as a magnetic probe, particularly for magnetic resonance imaging (MRI) of cells.

Background

MRI is a technique that allows whole body *in vivo* imaging in three dimensions at near-cellular (microscopic) resolution. In MRI a static magnetic field is applied to the object of interest while simultaneously or subsequently applying pulses of radio frequency-induced magnetization to change the distributions of the magnetic moments of protons in the object. The change in distribution of the magnetic moments of protons in the object from random to directed and their return to normal (random) constitute the MRI signal. Magnetic resonance (MR) contrast agents assist this return to normal by shortening T_1 and/or T_2 relaxation times.

In particular, MR image contrast is largely determined by the nuclear magnetic relaxation times of tissues. The longitudinal relaxation time T1 is defined as the time constant of the exponential recovery of proton spins to their equilibrium distribution along an applied field after a disturbance. The transverse relaxation time T2 is the time constant that describes the exponential loss of magnetization in a plane transverse to the

direction of the applied field, following a RF pulse that rotates the aligned magnetization into the transverse plane. MR contrast agents shorten the T1 and T2 relaxation times and their net effectiveness is expressed as relaxivity (R), which represents the reciprocal of the relaxation time per unit concentration of metal, with units of $mM^{-1}s^{-1}$.

Superparamagnetic iron oxide particles such as Fe_3O_4 and Fe_2O_3 particles are known MR contrast agents. However, such superparamagnetic particles by themselves (i.e., without a specialized treatment) are water insoluble and, thus, must be delivered for use in the form of an aqueous suspension of the particles. Conventional superparamagnetic iron oxide particles, such as dextran-coated particles, cannot be used in MRI of living cells since they have no affinity for cellular membranes.

Dendrimers are highly branched synthetic polymers that consist of three different building blocks – core, branching units and functional terminal groups. Various metallodendrimers have been described in the literature. According to one type of approach, metallodendrimers have been produced by complexing metal ions with a dendrimer followed by selective chemical reduction of the metal ions (see, e.g., Balogh et al., *Poly(Amidoamine) Dendrimer-Templated Nanocomposites. I. Synthesis of Zerovalent Copper Nanoclusters*, J. Am. Chem. Soc., 120, 7355-7356 (1998)). For example, aqueous suspensions of Au colloids in the 2-3 nm size regime were prepared by *in situ* reduction of $HAuCl_4$ in the presence of poly(amidoamine) dendrimers (Garcia et al, *Preparation and Characterization of Dendrimer-Gold Colloid Nanocomposites*, Anal. Chem. 71, 256-258 (1999)). Another approach has been to functionalize the terminal groups of dendrimers with a metal chelating agent that is then chelated to a metal ion (see, e.g., Wiener et al., *Dendrimer-Based Metal Chelates: A New Class of Magnetic Resonance Imaging Contrast Agents*, Magn. Reson. Med. 31, 1-8 (1994); and U.S. Patents No. 5,714,166 (Tomalia et al.) and 5,527,524 (Tomalia et al.)). For example, MR contrast agents have been reported that were formed by adding Gd^{3+} ions to a metal chelate-dendrimer conjugate (Wiener et al., *Dendrimer-Based Metal Chelates: A New Class of Magnetic Resonance Imaging Contrast Agents*; and

Bryant et al., *Synthesis and Relaxometry of High Generation (G=5,7, 9 and 10) PAMAM Dendrimer-DOTA-Gadolinium Chelates*, J. Magn. Reson. Imaging 9, 348-352 (1999)). In addition, U.S. Patents No. 5,714,166 and 5,527,524 describe chelation of ferric ions by a sodium propionate-terminated sixth generation poly(amidoamine) dendrimer.

Many therapeutic strategies, such as stem cell transplantation, are based upon introducing exogenous living cells or tissues into a patient or host. A problem common to all therapeutic strategies involving administration of exogenous cells is identifying and monitoring the cells in the host. It is currently difficult or impossible to monitor the location of such cells or tissues in the host after administration. It may also be difficult to establish the survival of these cells in the host. Currently available procedures to locate transplanted cells are invasive and destructive. This problem must be overcome before such cell therapies can achieve their full potential. Cellular therapy and diagnostics in humans would be advanced by a technique that can monitor their fate non-invasively and repeatedly, in order to take a momentary "snapshot" assessment of the cellular biodistribution at a particular given time point.

One class of cells that has been receiving substantial attention are human embryonic stem (ES) cells since they can now be isolated and propagated indefinitely in culture. They can be differentiated into virtually any cell type and have great therapeutic potential to replace or substitute defunct endogenous cell populations. In order to determine the history and fate of transplanted cells, including their migration *in vivo*, cells are currently labeled *ex vivo* using a vital dye (e.g. a fluorochrome), a thymidine analogue (e.g. BRDU), or a transfected gene (e.g. LacZ or GFP), which can be visualized using (immuno)histochemical procedures following tissue removal at a particular given time point.

Summary of the Disclosure

The disclosure provides a new class of magnetic probes that can achieve a high degree of intracellular magnetic labeling that is non-specific (i.e., not dependent on targeted membrane receptor binding) and that can be used on virtually any mammalian cell, including human multipotent stem cells. The magnetic probe could be used in cell tagging, as an MR contrast agent, cancer therapy hyperthermia, cellular therapy, magnetic guidance of cells, ultrasound imaging, microwave radiation and nuclear isotope imaging using ^{59}Fe preparations. A particularly useful application is as an MR contrast agent that enables non-invasive and repeated identification and monitoring of cells with a high degree of precision.

According to one disclosed embodiment, a magnetic probe is provided in an aqueous suspension that includes magnetic-responsive metal (oxyhydr)oxide particles stabilized by a dendrimer. It has been found that synthesizing or forming metal (oxyhydr)oxide particles in the presence of a dendrimer produces metal (oxyhydr)oxide particles that remain in a stable aqueous suspension for an extended period of time. One type of magnetic probe is an MR contrast agent that includes an iron oxide-dendrimer complex. A variant MR contrast agent is an aqueous suspension that includes iron oxide particles synthesized in the presence of a carboxy-terminated poly(amidoamine) dendrimer.

Various methods are also disclosed for using such magnetic probes. For example, there is provided a method of labeling living cells to render the cells magnetic resonance sensitive that includes contacting a metal (oxyhydr)oxide-dendrimer composition with the cells to be labeled and allowing the metal (oxyhydr)oxide-dendrimer composition to be internalized by the cell. This magnetic labeling renders the cells identifiable and distinguishable by MRI. In particular, an MR contrast agent can be used in the imaging of tissues, organs, cells, antigens, tumors and the blood pool

by labeling and detecting living cells in the object of interest. A wide variety of cells, including human neural and mesenchymal stem cells, can be very efficiently magnetically labeled while retaining their normal capability for differentiation.

A further aspect of labeling with the metal (oxyhydr)oxide-dendrimer composition involves detecting living cells in a host by contacting a metal (oxyhydr)oxide-dendrimer composition with the cells, allowing the metal (oxyhydr)oxide-dendrimer composition to be internalized by the cells, and imaging the host with magnetic resonance imaging so as to detect the cells. For example, using a rat brain transplantation model it has been demonstrated that magnetically labeled cells can be easily detected *in vivo*, with a good agreement between a traditional marker gene and the contrast on the MR images.

An additional disclosed embodiment includes a method of manipulating cells by contacting a magnetic-responsive metal (oxyhydr)oxide-dendrimer composition with the cells, allowing the magnetic-responsive metal (oxyhydr)oxide-dendrimer composition to be internalized by the cells, providing a suspension that includes the magnetically-tagged cells, and subjecting the magnetically-tagged cells to a magnetic field to attract the magnetically-tagged cells.

According to additional disclosed embodiments there are provided various chemical complexes. One example is an iron oxide-carboxylated dendrimer complex. A second example is an iron oxide-dendrimer complex wherein the iron oxide is bound to a dendrimer molecule at an outer external surface of the dendrimer molecule.

Another aspect of the disclosure is a water soluble aggregate that includes a metal (oxyhydr)oxide domain having more than one discrete particle; a dendrimer domain having more than one generally spherical dendrimer molecule, each molecule having a peripheral external surface; wherein the metal (oxyhydr)oxide domain is substantially confined within the dendrimer domain by the peripheral external surfaces of the dendrimer molecules.

According to a further disclosed embodiment there are provided various aqueous compositions that include metal (oxyhydr)oxide particles. One example is an aqueous suspension comprising metal (oxyhydr)oxide particles stabilized by a dendrimer. A second example is an aqueous suspension comprising metal (oxyhydr)oxide particles formed in the presence of a dendrimer. A third example is an aqueous mixture of ingredients comprising a ferrous or ferric ion-containing material and a dendrimer having terminal groups selected from carboxy, sulfono, sulfonato, phosphono and phosphonato. A fourth example is an aqueous suspension comprising iron oxide particles; and dendrimer molecules having terminal groups selected from carboxy, sulfono, sulfonato, phosphono and phosphonato.

Additional embodiments concern methods of making metal (oxyhydr)oxide-dendrimer compositions and aqueous suspensions that contain such compositions. One such method includes contacting a metal ion-containing material with a dendrimer having terminal groups selected from carboxy, sulfono, sulfonato, phosphono and phosphonato; and oxidizing or hydrolyzing the metal ion-containing material in the presence of the dendrimer. In another method a ferrous or ferric ion-containing material is contacted with a dendrimer, and the ferrous or ferric ion-containing material is oxidized in the presence of the dendrimer to form an iron oxide. A third disclosed method for synthesizing metal oxide nanoparticles is *in situ* oxidation of a metal ion-containing material in the presence of a dendrimer. A fourth disclosed method of making an iron oxide-dendrimer complex includes adding at least one of Fe^{2+} or Fe^{3+} ions to an aqueous suspension of carboxy-terminated poly(amidoamine) dendrimer to form an intermediate complex and oxidizing the intermediate complex.

One example of a method for making an aqueous suspension that includes superparamagnetic iron oxide particles includes mixing a ferrous or ferric ion-containing material with a carboxy-terminated dendrimer, and oxidizing the ferrous or ferric ion-containing material in the presence of the dendrimer to form suspended superparamagnetic iron oxide particles. Another example of a method for making an aqueous suspension that includes superparamagnetic iron oxide particles involves

mixing together in an aqueous medium the following ingredients: (a) a carboxy-terminated dendrimer; (b) a material that includes at least one of Fe^{2+} or Fe^{3+} ions; and (c) an oxidizing agent.

The disclosed magnetic probes offer superior MRI contrast-enhancing capabilities. For example, the metal (oxyhydr)oxide-dendrimer composition of the invention exhibits unusually high T2 MR relaxivities of solvent water protons. In particular, the superparamagnetic metal (oxyhydr)oxide-dendrimer compositions may have a saturation magnetization of greater than about 90 emu g/metal atom and T2 relaxivity of at least about 200 s^{-1}/mM metal atom at 37°C.

Moreover, the magnetic probes show a high, non-specific affinity for cellular membranes. For example, the metal oxide-dendrimer composition induces sufficient MR contrast at cell incubation doses as low as about 1 μg Fe atom/ml. Thus, these magnetic probes offer the opportunity to label mammalian (stem) cells regardless of their origin or animal species thereby creating the possibility of MR tracking of a wide variety of cell transplants.

A further advantage is that the dendrimer stabilizes superparamagnetic iron oxide particles in aqueous suspensions at concentrations where untreated iron oxide particles precipitate. For example, superparamagnetic iron oxide particles may be suspended in an aqueous suspension at a concentration of up to about 1 M Fe. Moreover, an aqueous suspension that includes the iron oxide-dendrimer complex is robust and easy to use.

The foregoing features and advantages will become more apparent from the following detailed description of several embodiments that proceeds with reference to the accompanying figures.

Brief Description of the Drawings

Certain embodiments are described below with reference to the following figures:

Figure 1 is a schematic representation of one disclosed embodiment of a metal oxide- dendrimer composition synthesis;

Figure 2 is a graph depicting titration of H^+ generated by oxidative hydrolysis of Fe(II) in a pH-stat experiment in accordance with a disclosed embodiment wherein (○) represents adding reactants over 1 minute, (△) represents adding reactants over 3.5 minutes and (◊) represents adding reactants over 20 minutes;

Figure 3 is a graph depicting the change in absorbance during a synthesis in accordance with a disclosed embodiment, wherein each spectrum line is generated one minute apart;

Figures 4A-4D are transmission electron micrographs of metal oxide-dendrimer compositions in accordance with a disclosed embodiment;

Figures 5A-5D are electron diffraction data from an aggregate in accordance with a disclosed embodiment;

Figure 6 is a graph depicting size exclusion chromatography results of a metal oxide-dendrimer composition synthesized with one minute reactant addition in accordance with a disclosed embodiment wherein curve A is for a sample removed from the reaction after 60 minutes, curve B is a sample removed after 10 minutes and curve C is a sample removed after 5 minutes;

Figure 7A is a spectrum of metal oxide-dendrimer composition synthesized in accordance with a disclosed embodiment wherein (○) represents adding reactants over a 1 minute period, (◊) represents adding reactants over a 7.5 minutes period; and (△) represents adding reactants over a 20 minutes period;

Figure 7B is a spectrograph of metal oxide-dendrimer composition synthesized in accordance with a disclosed embodiment wherein (o) represents the change in absorbance at 400 nm and (Δ) represents the change in absorbance at 800 nm for a metal oxide-dendrimer composition synthesized with a one minute reactant addition time and a 60 minutes subsequent heating or annealing time;

Figures 8A-8D are transmission electron micrographs of metal oxide-dendrimer compositions in accordance with a disclosed embodiment wherein A is reactant addition over 1 minute, B is reactant addition over 3.5 minutes, C is reactant addition over 15 minutes and D is reactant addition over 20 minutes;

Figures 9A-9D are transmission electron micrographs of metal oxide-dendrimer compositions synthesized with one minute reactant addition in accordance with a disclosed embodiment wherein A is removal from reaction after 5 minutes, B is removal from reaction after 30 minutes and C is removal from reaction after 60 minutes and D is a 100 Fe loading factor sample prepared by 20 minutes reactant addition and 40 minutes annealing time;

Figure 10 is a graph depicting the magnetization of metal oxide-dendrimer compositions synthesized in accordance with a disclosed embodiment wherein (◊) represents a 100 Fe loading factor sample prepared by 20 minutes reactant addition and 40 minutes annealing time, (Δ) represents 1 minute reactant addition and 60 minutes annealing and (o) represents 1 minute reactant addition and 10 minutes annealing;

Figures 11A and 11B are graphs depicting T1 and T2 relaxivities, respectively, for metal oxide-dendrimer composition samples synthesized in accordance with a disclosed embodiment of the invention and measured at 3°C (open symbols) and 37°C (solid symbols) wherein (□) represents a 100 Fe loading factor sample synthesized by reactant addition of 20 minutes and 40 minutes annealing, (Δ) represents a sample synthesized by 1 minute reactant addition and 60 minutes annealing, and (o) represents a sample synthesized by 1 minute reactant addition and removal from the reaction after 10 minutes;

Figures 12A-F are micrographs of cells labeled with a MR contrast agent in accordance with a disclosed embodiment, wherein (A) are HeLa human cervix carcinoma cells, (B) are GLC-28 human small cell lung carcinoma cells, (C) are CG-4 rat oligodendrocyte progenitor cells, (D) are 3T3 mouse fibroblasts, and (E,F) are C2C12 mouse muscle progenitor cells ((A)-(E) utilize non-enhanced Prussian blue stains and (F) utilizes DAB-enhanced Prussian Blue stains);

Figures 13A-C are graphs depicting the T1 (A) and T2 (B) relaxation rates for cells labeled with a metal oxide-dendrimer composition in accordance with a disclosed embodiment and non-labeled cells and the corresponding total iron content (C);

Figures 14A-D depict separation of cells labeled in accordance with a disclosed embodiment, Figure 14E shows washed cells used in the cell separation with a dark pellet indicating magnetically labeled cells, and Figure 14F shows a NdFeB permanent magnet used for the cell separation;

Figures 15A, B and D-F are micrographs of cells labeled with a MR contrast agent in accordance with a disclosed embodiment, wherein (A) and (B) are (DAB-enhanced) Prussian Blue stains of a labeled human neural stem cells, (D) is an anti-neuron immunostaining of labeled human neural stem cells, (E) is a (DAB-enhanced) Prussian Blue stain of a labeled neural stem cell-derived rat oligodendroglial progenitor and (F) is a Prussian Blue stain of a labeled trypsinized human multipotent mesenchymal stem cells and Figure 15C is a (DAB-enhanced) Prussian Blue stain of unlabeled human neural stem cells;

Figures 16A-16D are MR images (1.5 T) of human multipotent mesenchymal stem cells, wherein (A) are unlabeled cells, and (B)-(D) are cells labeled with 1 μg Fe/ml, 4 μg Fe/ml, and 10 μg Fe/ml, respectively, of an MR contrast agent in accordance with a disclosed embodiment;

Figure 17A is an *in vivo* MR image of *les* rat stem cells 42 days post intraventricular transplantation of magnetically labeled and LacZ-transfected, neural stem cell-derived oligodendroglial progenitors and Figure 17B is an X-gel staining of corresponding gross specimen; and

Figures 18A and B are *in vivo* MR images of a small cell lung tumor at 20 days following implantation (A) and 27 days following implantation (B) of cells labeled in accordance with a disclosed embodiment; Figures 18C and 18D show *ex vivo* MR images of a small cell lung tumor, and Figure 18E shows a micrograph of the correlating (DAB-enhanced) Prussian Blue staining for the image in Figure 18D.

Detailed Description of Several Embodiments

The following definitions are provided for ease of understanding and to guide those of ordinary skill in the art in the practice of the embodiments.

"Ferrous or ferric ion-containing material" denotes a material such as a compound or molecule that includes a ferrous or ferric ion.

"Metal (oxyhydr)oxide" denotes a metal oxide produced by oxidation of a transition metal ion or a metal oxyhydroxide produced by hydrolysis of a lanthanide metal ion.

"Metal (oxyhydr)oxide-dendrimer composition" refers generically to, and is inclusive of, complexes, aggregates, nanocomposites or aqueous suspensions.

It has been found that a dendrimer may be used for controlling the synthesis of iron oxides particles. Certain dendrimers lead to the formation of well-defined superparamagnetic iron oxide nanoparticles. In particular, oxidative hydrolysis at the dendrimer-suspension interface, under controlled and mild synthetic conditions, leads to the formation of composite assemblies of dendrimer and inorganic mineral.

Figure 1 depicts a particular embodiment of a synthesis scheme for making a water soluble aggregate **1** that is a nanocomposite or assembly of metal oxide particles **2** (in this case γ-Fe_2O_3) and dendrimer molecules **3**. Although not bound by any theory, it is believed that the dendrimer molecules **3** constitute an oligomeric envelope that substantially encompass or surround the metal oxide particles **1**. In a particular example, the water soluble aggregate or nanocomposite **1** may have a diameter of at

least about 15 nm with an illustrative range of about 15 to about 35 nm and a total molecular weight of about 100 Kdaltons to about 1000 Kdaltons.

A metal ion-containing material can be used as a starting material. The metal ion-containing material is contacted or mixed with at least one dendrimer. The metal ion-containing material/dendrimer mixture is subjected to oxidation or hydrolysis conditions leading to the formation of an oxide or oxyhydroxide of the metal atom in the metal ion-containing material. In other words, the metal (oxyhydr)oxide is formed *in situ* in the presence of the dendrimer. According to certain embodiments, the reaction could also be considered a "mineralization" since the resulting metal (oxyhydr)oxide may be a crystalline mineral. An oxidizing agent may be used for effecting the oxidation and/or hydrolysis. Deaerated starting materials may be employed.

The sequence for adding together the metal ion-containing material, dendrimer and oxidizing agent is not critical and may vary. For example, the metal ion-containing material and the dendrimer may be first mixed together followed by the addition of the oxidizing agent or the metal ion-containing material and the oxidizing agent may be added simultaneously to the dendrimer. The starting materials also may be added together over a relatively short time period such as about one minute or the addition may be more controlled for a longer time period. For example, the oxidizing agent may be added over a period of about 1 to about 60 minutes. A slower rate of addition appears to lead to the development of a more fully crystalline metal (oxyhydr)oxide particle.

The relative amounts of the metal ion-containing material, dendrimer and oxidizing agent may vary. For example, the amount of metal ion-containing material relative to the dendrimer may range from about 10 to about 200, preferably from about 100 to about 200, molar ratio of metal ion:dendrimer. According to one embodiment, the oxidizing agent is added in an approximately stoichiometric amount relative to the metal molar content of the metal ion-containing material.

The conditions of the reaction are sufficient to oxidize and/or hydrolyze a substantial portion of the metal ions in the metal ion-containing material. Substantially 100% of the metal ions can be oxidized and/or hydrolyzed. Heat may be added during the reaction to assist in the oxidation, hydrolysis and/or mineralization. For example, once the oxidizing agent is added to the reaction mixture, the reaction mixture may be heated at a temperature of at least about 50, preferably about 60, °C for about 10 to about 120, preferably about 30 to about 60, minutes. The oxidation reaction may be limited to about 60 minutes or less. The pH of the reaction mixture also may be controlled in a range of about 8.0 to about 10.0, for example about 8.5 to about 9.0. This pH range indicates that OH^- is present that causes hydrolysis of the metal-ion containing material. The oxidation/hydrolysis can be performed in an aqueous medium under anaerobic conditions. Alternatively, the oxidation/hydrolysis can be performed in an aqueous medium under ambient atmosphere such that the O_2 in the atmosphere acts as the oxidizing agent.

Although not bound by any theory, it is believed that the metal (oxyhydr)oxide particle formation occurs in intermediate stages as shown in Figure 1. A metal ion-containing material, dendrimer and oxidizing agent are mixed together under conditions to oxidize the metal ion-containing material. Metal oxide particles **4** begin to form initially and simultaneously bond to an external outer surface **5** of the dendrimer molecule. The result is a metal oxide-dendrimer complex. It is believed that the terminal carboxy groups initially bind and sequester the metal ion, with the dendrimer acting here as a nucleation site for iron oxide synthesis. An intermediate complex **6** is shown in Figure 1. Such an intermediate complex **6** has an average diameter of about 7 to about 12 nm. Reports suggest that individual dendrimer molecules are approximately 4-5 nm in diameter (Tan et al., Polymer, 40, 2537-2545 (1999); Nisato et al. Macromolecules, 32, 5895-5900 (1999)), roughly half the size of the iron oxide particles produced in this synthesis. Thus, it appears that the mineral particles are not entrapped within the dendrimer but rather grow from the dendrimer surface. However,

it may be possible for dendrimers higher than 4.5 generation dendrimers to grow metal (oxyhydr)oxide particles at least partially within the dendritic spherical architecture. According to a particular embodiment utilizing a carboxy-terminated dendrimer, the highly charged carboxylate interface provides a surface very favorable for the stabilization of iron oxides. Maghemite nanoparticles appear to form from an initial green-rust like material which in our experiments shows a different particle size very early on in the reaction. The particle size of this material rapidly changes, suggesting an aggregation of dendrimer molecules around the embryonic particle that then matures into its final mineral form. This particle growth is borne out by the size exclusion chromatography, TEM, magnetometry and relaxivity data provided below in the examples.

Dendrimers have a three-dimensional architecture with a core, a branching unit region, and terminal groups located at the outer surface or periphery of the three-dimensional architecture. Dendrimers can be spheroid-shaped, cylindrical or rod-shaped, ellipsoid-shaped or mushroom-shaped. An example of such a dendrimer is a so-called "starburst" dendrimer having concentric dendritic tiers around an initiator core. Dendrimers are named according to "generations" which indicate the size of the dendrimer. Generation 4.0 or higher dendrimers are used according to particular embodiments. Mixtures of different dendrimers may be utilized.

Examples of dendrimers that may be useful include those that have cores of ammonia or 1,4-diaminobutane such as poly(amidoamine) (PAMAM) or poly(propyleneimine) (PPI) dendrimers. PAMAM or PPI dendrimers may be modified by techniques known in the art to include specific functional outer surface terminal groups other than amino or methyl ester (in the case of PAMAM). For example, the methyl ester groups of PAMAM may be converted to carboxyl groups via hydrolysis (see, e.g. U.S. Patent No. 5,527,524). Dendrimers having carboxy, sulfono, sulfonato, phosphono and phosphonato functional groups, including the salts or esters thereof, as the outer surface terminal groups may be particularly useful for stabilization of metal oxide particles. Carboxy-terminated PAMAM dendrimers (generation 4.5) such as a

sodium carboxylate salt or a methyl ester carboxylate are one example of a useful dendrimer. The dendrimers used may be aqueous solutions.

The metal ion-containing material used as a starting material may be in the form of a metal salt. The metal atom in the metal ion-containing materials may include transition or lanthanide metal ions. Illustrative transition or lanthanide metal ions include iron, cobalt, gadolinium, europium and manganese. Particularly exemplary embodiments include as the starting materials the NO_3^-, SO_4^{2-}, Cl^-, Br^-, I^-, acetate or oxalate salts of transition or lanthanide metals. For example, useful ferrous or ferric ion-containing materials include $(NH_4)_2Fe(SO_4)$, $FeCl_2$ and $FeSO_4$. Mixtures of different metal ion-containing materials may be utilized. In the case of a transition metal ion as the starting material, the transition metal ion is oxidized to its corresponding metal oxide. In the case of a lanthanide metal ion as the starting material, the lanthanide metal ion is oxidized to its corresponding metal oxyhydroxide.

Oxidizing agents that may be used in connection with the invention are those that will oxidize a metal ion to its higher oxidation state under the disclosed reaction conditions. Illustrative oxidizing agents include $(CH_3)_3NO$, H_2O_2, KIO_3, and O_2. Mixtures of different oxidizing agents may be utilized.

Optionally, a metal chelating agent may be added to the reaction or product mixture to chelate with any non-oxidized metal ion-containing material. The resulting chelated metal is then removed from the product mixture via known separation techniques such as dialysis, gel filtration, ultrafiltration or ion exchange. Thus, the aqueous suspension of the metal (oxyhydr)oxide-dendrimer complex is substantially free of any metal chelating agents. Preferably, the metal chelating agent is added after the oxidation has been completed as determined by monitoring of the pH and visible-UV spectrum. Exemplary metal chelating agents include citrates, diethylenetriaminepentaacetic acid (DTPA), ethylenediaminetetraacetic acid (EDTA), and acetates. Mixtures of different metal chelating agents may be utilized.

The magnetic-responsive metal (oxyhydr)oxide particles may be paramagnetic, ferrimagnetic, superparamagnetic or anti-ferromagnetic.

According to one useful embodiment the metal oxide particles are superparamagnetic, particularly iron oxide superparamagnetic particles. Examples of such superparamagnetic iron oxide particles include magnetite and maghemite (i.e., γ-Fe_2O_3). These particles possess a large ferrimagnetic moment that, because of the small crystal size, is free to align with an applied magnetic field (i.e., there is no hysteresis). The aligned magnetization then creates microscopic field gradients that dephase nearby protons and shorten the T2 NMR relaxation time, over and beyond the usual dipole-dipole relaxation mechanism that affects both T1 and T2 relaxation times.

The particle aggregates or nanocomposites maintain their suspension in water over a wide range of conditions for extended periods of time. For example, the aqueous suspensions of the aggregates or nanocomposites are stable for at least 12 months. As used herein, a "stable" aqueous suspension means that substantially no precipitate is formed as the suspension rests in a substantially stationary position in the presence of atmospheric oxygen. Although not bound by any theory, it is believed that the dendrimer contributes initially to forming the nanocomposites but limits additional interparticle aggregation. If the particles would continue aggregating, the aggregate would eventually reach a critical size at which it would precipitate. A highly colloidally suspendable nanocomposite material results from the limited aggregation. The dendrimer could act to stabilize the particles in a number of ways. By virtue of the high charge density of its functionalized surface the dendrimer is expected to assist nucleation through stabilization of embryonic aggregates. This would result in the formation of small particles. In addition, dendrimer-particle interactions might act to passivate the metal oxide surface and thus limit particle growth as well as block the interparticle interaction and aggregation as shown schematically in Figure 1.

Particle aggregation beyond the critical size and resulting precipitation, however, can be reversibly induced by the application of a strong magnetic field gradient to the suspension. Upon removal of the magnetic field, complete and immediate suspension is restored. Such behavior is characteristic of a superparamagnetic material having no remnant magnetization.

As described above, the metal (oxyhydr)oxide-dendrimer compositions can be used as magnetic probes. One particular embodiment includes labeling living cells with the metal (oxyhydr)oxide-dendrimer composition to render the cells MR sensitive. Such magnetically labeled cells may be prepared by simple incubation of cells with the metal (oxyhydr)oxide-dendrimer composition in cell cultures. Suitable cell incubation techniques are well known. For example, a cell of interest can be cultured in a standard media that includes the iron oxide-dendrimer composition at a dose ranging from about 1 to about 25 μg Fe/ml. Alternatively, the metal (oxyhydr)oxide-dendrimer composition can be injected into tumors and other areas to label cells *in situ* or by injecting into blood vessels, ventricles or other brain or body cavities.

The metal (oxyhydr)oxide-dendrimer composition may be internalized by a cell by binding to the cell surface with subsequent entrance into the cytoplasm. Although not bound by any theory, it is believed that the metal (oxyhydr)oxide-dendrimer composition is internalized by a cell via a non-specific membrane adsorption process with subsequent intracellular localization in endosomes.

The magnetically labeled cells may be exogenously applied to a host and monitored within the host using MRI. For example, such cells may be injected or otherwise applied to the host.

Another option is to label cells in the host *in situ* so as to allow labeling of structures within the host. This would allow monitoring of labeled structures and cells. For example, tumors could be labeled to monitor effectiveness of treatment and follow metastasis. The cells can be labeled *in situ* for therapeutic, diagnostic, or experimental purposes. Another embodiment encompasses infusing the magnetic probes into such areas as tumors, so that the growth, metastasis, or regression of the tumor can be monitored. Such a procedure could be part of a treatment protocol to monitor disease progress.

The living cells for labeling and detection in accordance with the invention are those that are of therapeutic, diagnostic, or experimental value when introduced into a patient or host. The term "cell" is understood to mean embryonic, fetal, pediatric, or

adult cells or tissues, including but not limited to, stem cells, precursors cells, and progenitor cells. It is also understood that the term "cells" encompasses virus particles and bacteria. The term 'host' can mean any mammalian patient or experimental subject, including human patients or subjects. The living cells of the current invention can be bone marrow cells, hematopoietic cells, tumor cells, lymphocytes, leukocytes, granulocytes, hepatocytes, monocytes, macrophages, fibroblasts, neural cells, mesenchymal stem cells, neural stem cells, and combinations thereof. It is understood that the term "neural cells" includes neurons and neuroglia. The term "neuron" encompasses central and peripheral neurons, central nervous system neurons, and neuropithelial cells. The term "neuroglia" encompasses oligodendrocytes, astrocytes, ependymal cells, microglia, stellate cells, Schwann cells, and neurilemma. Preferably the cells are neural stem cells or oligodendrocyte progenitors. Oligodendrocytes are glial cells of the central nervous system, which form sheaths around nerve fibers and capsules around nerve cell bodies.

The cells can be applied to the host to cure or diagnose a disease or to supply cell type that is lacking or deficient in the host. The cells can also provide a drug or substance that is needed in the host for diagnostic, therapeutic, or experimental purposes. Cells can be immune cells to specific proteins in the host's body, such as proteins found in malignant tissue or molecules associated with a disease state such as bacterial or veal proteins or glycoproteins.

In one embodiment the cells can be stem cells. Stem cells are cells that retain their ability to divide and to differentiate into specialized mature cells. Preferably the cells are multipotent cells from the nervous which retain their ability to differentiate into oligodendrocytes. Oligodendrocytes are cells that mylinate or provide protective sheaths to neurons and axons in the central nervous system.

In another aspect the cells are carcinoma cells. Such cells are neoplastic and divide indefinitely. Preferred tumor cells is small cell carcinoma cells. Labeling such cells *in vitro* is an experimental tool to study how such cells behave in experimental

conditions. Cancer cells can also be labeled *in vivo* to allow clinical investigators to track possible metastases.

In a further aspect, the method includes applying such cells, which are labeled with the MR contrast agent, for any therapeutic, diagnostic, or experimental purposes. The cells can be dispersed, or can be part of a tissue or organ or labeled cells can be applied to any tissue or organ after the cells are labeled. The cells can be directly applied to the area to be treated or studied by means of surgery or injection into the circulation or injection into a structure, organ, or body cavity *in situ*. When cells are integrated *ex vivo* into a tissue or organ, such tissue or organ can then be surgically applied or transplanted into a host. Preferably, the cells are applied directly to a body structure. Most preferably the cells are applied to the central nervous system.

A further embodiment involves using MRI to monitor the movement, disposition and survival of the cells in the host. When cells are used that are immune cells, which react with a component of a disease process in the host, MRI monitoring can be used diagnostically to locate the cells attached to the disease process in the host. Immune cells are understood to encompass lymphoid or myeloid hematopoietic cells. Examples of such disease processes are malignant and metastatic diseases, degenerative diseases and infectious diseases. The cells can be used experimentally, in animal hosts, to study the development of disease as a basis for designing therapeutic strategies. Tumor or neoplastic cells can be applied to animals as an experimental technique to study the behavior of neoplastic growth and metastasis in the organism.

Cells can be used to replace injured or diseased cells in the host, examples are diseases of the nervous system, injuries to the nervous system, injuries or diseases of bone, muscle, heart, circulation, internal organs, skin, interstitial tissue, mucosa, lungs, and gastrointestinal tract. When cells are used in this way, the host can be scanned with MRI to establish the location of the cells, the movement or migration if any of the cells, and the survival of the cells. The host can be scanned with MRI as frequently and during as long a period of time as required or desirable to monitor the cells. Cells that are loaded with therapeutic vectors can be monitored as necessary to establish their

migration if any in the host and to establish their continued survival and ability to produce therapeutic proteins or drugs in the host.

The specific examples described below are for illustrative purposes and should not be considered as limiting the scope of this invention.

Example A – Synthesis and Characterization

A carboxy-terminated dendrimer (gen 4.5, commercially available from Dendritech,) (10mg, $4x10^{-4}$ mmoles) was added to the reaction vessel and the dendrimer solvent (methanol) was removed by a stream of N_2 for 20 minutes. Following this, 30mL of deaerated H_2O (0.1M NaCl, pH 8.5) was added and further deaerated by bubbling N_2 for 20 minutes and maintained under N_2 during the reaction. For an Fe loading (or molar ratio of Fe:dendrimer) of 100 , deaerated suspensions of $(NH_4)_2Fe(SO_4)$ (25mM, 1.58mL, $4x10^{-2}$mmoles) and the 2 electron oxidant Me_3NO (i.e., $(CH_3)_3NO$) (25mM, 1.58mL) were added at a fixed rate via syringe pump. Fe(II) and Me_3NO suspensions were added at rates of 1.58mL/min, 0.45mL/min, 0.21mL/min, or 0.105 mL/min. For syntheses of metal oxide-dendrimer compositions with a Fe loading factor of 50, 0.79mL of $(NH_4)_2Fe(SO_4)$ (25mM) and 0.79mL of Me_3NO (25mM) were used. For Fe loading factors of 200, 3.16 mL of $(NH_4)_2Fe(SO_4)$ (25mM) and 3.16 mL of Me_3NO (25mM) were used. The reaction was performed using Fe:dendrimer ratios of 50, 100, 200, and 250 with reagents (i.e., $(NH_4)_2Fe(SO_4)$ and Me_3NO) being added over the course of 20 minutes followed by an additional 40 minutes of heating at 65°C. The suspension pH was maintained by titration of the H^+ in a pH-Stat experiment using a Brinkmann 718 autotitrator. This eliminated the need to use contaminating buffers and allowed monitoring of the progress of the reaction. The change in pH was due in part to the acidic nature of the Fe(II) added and also due to H^+ generated by the oxidative hydrolysis reaction to form the iron oxide mineral. After addition and removal of the Na_2citrate, the completion of the reaction, Na_2 citrate was added (to 35mM) to remove any non-bound metal ion. After the reaction, the metal

oxide-dendrimer composition product was dialyzed exhaustively against double distilled H_2O and concentrated 50 fold by ultrafiltration (Amicon) using a 100kDa M_w cutoff filter.

The reaction proceeded from a colorless suspension through an intermediate green phase and finally to a brown suspension, consistent with proposed pathways for the formation of the iron oxide phase magnetite or maghemite.

No bulk precipitate was observed in the reaction performed in the presence of the carboxylated dendrimer (gen 4.5) at Fe: dendrimer ratios up to 200. These materials were quite stable to a wide range of conditions (pH of 4 to 11 and temperatures up to 100°C) for short periods of time without loss of solubility. In control reactions, in the absence of the dendrimer, the reaction mixture rapidly formed a dark brown precipitate of maghemite.

Metal oxide-dendrimer composition synthesized with a loading factor of 100 gave the most consistent and reproducible particle formation and this stoichiometry has been used in Example B below.

The results of monitoring the reaction and the properties of the resulting product (and the techniques for performing such analysis) are described below.

Attenuated total reflectance infrared spectroscopy (ATR-FTIR) of the reaction to form the metal oxide-dendrimer compositions was accomplished using a liquid flow cell (commercially available from Pike Technologies) with a ZnSe ATR crystal mounted into a Nicolet (Protégé 460) infrared spectrometer. During the reaction, liquid was pumped from the reaction vessel to the ATR flow cell at a rate of 200μL/min and 64 scans were collected.

Size exclusion chromatography was performed using a column packed with TOYOPEARL 75-F resin (commercially available from Supelco) and run on a BioLogic HR (commercially available from BioRad) medium pressure chromatography system. The flowrate in all experiments was 0.5mL/min and the background buffer was 100mM Tris, 150mM NaCl pH 7.5. Sample elution was monitored either at 405nm or

280nm. The column was calibrated with large molecular weight standards of tobacco mosaic virus, cowpea chlorotic mottle virus, blue dextran, and ferritin.

The Fe content was assessed using a Ferrozin-based spectrophotometric assay. X-ray powder diffraction data was collected on a Rigaku dMax2 diffractometer and indexed using JCPDS diffraction files. It is noted that maghemite (γ-Fe_2O_3, a=8.338Å) and magnetite (Fe_3O_4, a=8.377 Å) cannot be unambiguously distinguished per this assay technique because of the similarity in the lattice parameters. Samples were prepared for X-ray diffraction by extensive dialysis against double distilled H_2O and subsequently dried under vacuum.

The metal oxide-dendrimer composition nanoparticles were imaged using transmission electron microscopy (TEM) using a Phillips 420 at 120kV with an Kevex energy dispersive X-ray analysis system. High resolution TEM data were collected on a JEOL 4000FX microscope operating at 400 kV.

For SQUID magnetometry 200 µl suspensions of metal oxide-dendrimer composition containing 62-662 µg Fe were placed in a superconducting quantum interference device (SQUID) susceptometer (commercially available from Quantum Design). The magnetization was then measured over a field range –3 to +3 Teslas (-$3x10^4$ Oe to +$3x10^4$Oe) and at a temperature of 300 K. A sample holder correction was applied (subtraction of diamagnetic susceptibility) and moment calibration was performed using palladium and nickel standards. The measured magnetization was divided by the amount of iron, to yield specific magnetization (emu/g Fe). Thermal demagnetization curves were obtained in the temperature range from 6-240 K after the metal oxide-dendrimer composition suspensions were initially frozen at 240 K. Samples were cooled in zero field to 5 K, given a saturation remanence in a field of 2.5 T, and then measured in approximately zero field at 2 K intervals up to 240 K. The median blocking temperature of the system is taken as the temperature where 50% of the initial remanence has decayed.

Metal oxide-dendrimer composition samples for NMR relaxometry were adjusted to concentrations in the range 0.1-1.0 mM Fe. T1 and T2 were measured on a custom-designed variable-field relaxometer (available from Southwest Research Institute) at temperatures of 3 and 37 °C. For T1, 32 saturation-recovery sequences were used; T2 was measured with a CPMG sequence of 500 echoes with interecho time TE = 2 ms and 10 ms. The range of field strengths was 0.05-1.5 Tesla. The data were converted to relaxation rates (reciprocals of *T1* and *T2*); the background suspension contribution was then subtracted and the result divided by the iron concentration to obtain relaxivity values (s^{-1}/mM Fe).

The reaction to form the stable metal oxide-dendrimer composition with the generation 4.5 carboxy terminated dendrimers was investigated over a range of conditions including Fe:dendrimer stoichiometry (loading factor), rate of reagent (Fe and oxidant) addition, as well as the length of annealing (i.e., heating) time in suspension after the addition of reagents.

The oxidative hydrolysis reactions were monitored through the release of protons that were titrated dynamically to maintain the reaction at pH 8.5. The titration profiles for reagent addition over 1 minute, 7.5 minutes and 20 minutes are shown in figure 2. As shown in Figure 2, the oxidative hydrolysis reaction (followed by dynamic titration of H^+ generated during the course of the reaction in a pH-stat experiment) occurred rapidly, on the timescale of the reagent addition. A slow additional release of H^+ could be noted after complete addition of Fe(II) and oxidant. In this way reaction progress was monitored and approximately 2 equivalents of H^+ were generated per Fe(II) as expected for the oxidative hydrolysis of a ferric oxyhydroxide. In all cases the rate of reagent addition is clearly rate determining. Upon addition of Fe(II) and oxidant there is an oxidative hydrolysis reaction which results in the liberation of protons according to reaction 1. The reactions gave consistent values of 2.2±0.2 protons liberated per Fe. Figure 2 shows the release of protons.

$$2Fe^{2+}(aq) + (CH_3)_3NO \longrightarrow \gamma\text{-}Fe_2O_3(s) + (CH_3)_3N + 4H^+ \qquad \text{(rxn. 1)}$$

When followed spectrophotometrically (see Fig. 3), one can correlate the appearance of the green-colored intermediate with the rapid proton release from the initial oxidative hydrolysis. The appearance of the green color was followed by a slow transformation into the final mineral product having a characteristic broad visible absorbance consistent with the formation of the brown iron oxide.

The metal ion-containing mineral particles isolated from these reactions were imaged by transmission electron microscopy (TEM) which revealed fairly homogeneous particle sizes apparently aggregated into clusters (see Fig. 4). It is suspected that these aggregates are largely the result of drying down effects in the TEM sample preparation. Information regarding the individual mineral particles could be obtained due to the high contrast of the mineral particles. At low loading factors (50 Fe per dendrimer) particles having average diameter of 8.5nm (σ = 5nm) were observed (Fig. 4A). Samples synthesized with loading factor of 50 but under reaction conditions with two-fold higher dendrimer concentrations produced virtually identical particles (Fig 4B). At a loading factor of 100 the particles were of highly consistent morphology with an average diameter of 9.5 nm (σ= 5nm) as shown in Figure 4C. At higher loading factors (200 Fe per dendrimer) particles of similar average diameter (11nm, σ= 8nm) were observed but with a larger size distribution (Figure 4D). As shown by the electron micrograph the metal oxide-dendrimer composition samples synthesized at a loading factor of 200 show far less well-defined particles and there is evidence for secondary nucleation in that many small particles are observed. Electron diffraction collected from the mineralized dendrimers (Fig. 5A) revealed a powder pattern also consistent with a cubic iron oxide phase of magnetite or maghemite (d=2.96Å(220), 2.54Å(311), 1.62Å(511), 1.49Å(440), 1.09Å(553)). High resolution TEM (HRTEM) indicated the single crystal nature of the mineral particles; {111} (4.85Å) lattice fringes could be identified and measured at 71° (Figure 5B). As shown in Figure 5C precipitates formed in control reactions, which had been exhaustively dialyzed against ddH_2O, could be

identified as the mineral phase maghemite on the basis of the X-ray powder diffraction (Fig 5D) by comparison with powder diffraction standards.

These mineralized dendrimer samples could be isolated and purified using size exclusion chromatography and characterized on the basis of their elution behavior, specifically their retention times. Prior to chromatography, the metal oxide-dendrimer composition samples were concentrated 50-fold by ultrafiltration (100,000 mw cut-off). Concentration of the material in this way did not result in any loss of product through precipitation or passage through the ultrafilration membrane. The concentrated and unconcentrated material were indistinguishable by size exclusion chromatography. As shown in Figure 6, suspensions of the mineralized dendrimer eluted cleanly in a single peak on size exclusion chromatography and were monitored by the visible absorption of the iron oxide at 405nm. When samples, made at different rates of reagent addition and Fe loading factors, were chromatographed virtually identical elution profiles were obtained. Using a size exclusion chromatography resin with $5.0x10^7$ M_w exclusion limit (TOYOPEARL HW 75F) allowed analysis of the metal oxide-dendrimer compositions and roughly determine their suspension particle size. Elution profiles of the metal oxide-dendrimer composition samples were compared to the elution of high molecular weight standards of tobacco mosaic virus (M_w $40x10^6$), cowpea chlorotic mottle virus (M_w $3.5x10^6$), blue dextran (mw $2x10^6$) and ferritin (M_w $4.5x10^5$). The elution profile of the metal oxide-dendrimer composition lies between that of the cowpea chlorotic mottle virus and blue dextran. This suggests that the metal oxide-dendrimer composite aggregate structure lays in the 20-30nm diameter range, possibly comprising more then a single mineral and dendrimer particle but almost certainly not the extensively aggregated material that the TEM images suggest.

The oxidation reactions were monitored by changes in the absorption spectrum in the UV-visible range and the resulting reaction profile is depicted in Figure 7. On the timescale of reagent addition there was a commensurate increase in the region of the spectrum associated with the formation of a μ-ferric oxo species (350-450nm)

characteristic of these iron oxide materials. As shown in Figure 7A, when the rate of reagent addition was varied between 1 and 20 minutes there was an increase in the visible absorbance at 400nm, which plateaued soon after all the reactant had been added. This correlates with a change in color from colorless, at the beginning of the reaction, to deep green observed after addition of all the Fe(II) and oxidant. At reaction times much beyond the reagent addition there was a further increase in the visible absorbance as the reaction slowly changed color from green to brown (see Figure 7B). When the reactions were allowed to proceed for a total time of more than an hour there was a tendency of the reaction product to precipitate from suspension. Interestingly, the reaction products from reactions where the rate of reagent addition was varied from 1 to 30 minutes (but the same total reaction time) showed very little difference when imaged by transmission electron microscopy. As shown in Figure 8 the average particle size is roughly 9-10 nm in average diameter for all reactions with a 60 minutes total reaction time but rate of reagent addition varied between 1 and 20 minutes. In addition we could not resolve any significant differences in their elution behavior on size exclusion chromatography (Figure 6).

In order to investigate possible intermediate phases along the reaction path we undertook the synthesis of the metal oxide-dendrimer composition (100 Fe loading) using the most rapid rate of addition at which our auto-titrator could maintain pH-stat conditions (1 minute reagent addition). We had previously shown that the end products of a one minute reagent addition was morphologically not significantly different from the products synthesized under different rates of reagent addition. The reaction was monitored by the changes in the visible region of the spectrum. In addition, samples were removed at time intervals after the addition of Fe(II) and oxidant and the reaction quenched by addition of citrate. Samples were removed 5 minutes, and 10 minutes after addition of reagents and a final sample collected when the reaction was stopped after 60 minutes.

As shown in Figure 7B the visible absorbtion spectrum shows the development of a characteristic Fe-O absorbance (400nm) on the timescale of reagent addition (one minute) and plateaus after 3-4 minutes. After 10 minutes there was a further increase in the absorbance at 400nm that plateaued after 20-25 minutes and remained relatively flat until the end of the reaction. The second increase in visible absorbance was associated with the observed color change in the reaction from green to brown. It is of interest to note that the absorbance at the far end of the visible (800nm) exhibited the same rapid increase in absorbance as observed at 400nm reaching its maximum after roughly 5 minutes and remained fairly constant throughout the reaction. This absorbance behavior is consistent with the formation of an initial or intermediate complex that transforms into a final nanocomposite after roughly 30 minutes.

Transmission electron microscopy of samples isolated 60 minutes after the addition of reagents showed very well defined electron dense particles roughly 9.5 nm in diameter (Figure 9A). However, TEM images of the samples removed 5 and 10 minutes after reagent addition clearly show a marked difference from those collected at later times (Figures 9B and 9C). As shown in Figure 9, these samples had significantly less electron dense material and were more diffuse than samples from later on in the reaction. The identifiable particles were measured and found to have average diameters of 4.6 nm (σ=3 nm) and 5 nm (σ = 3 nm) respectively. This is consistent with the size exclusion chromatography characteristics described above in which the very earliest intermediate was significantly smaller in size than those at later times in the reaction.

The magnetic characteristics of samples taken from the oxidation reaction 10 minutes and 60 minutes after addition of reagent were investigated by SQUID magnetometry and the data is depicted in Figure 10. The magnetic behavior of these two materials were compared with a metal oxide-dendrimer composition sample synthesized using a 20 min addition of Fe(II) and oxidant and subsequently annealed for 40 minutes (Fe loading factor of 100). The magnetization as a function of magnetic field strength is shown in Figure 10. The data were fit using a Langevin function

assuming a uniform distribution of magnetic moments from 0 to μ_{max}. The magnetic particle moment of the 60 minute sample is nearly twice that of the 10 min sample, but not as high as the 100 Fe loading factor sample (Figure 10). The blocking temperature, above which the particle exhibits superparamagnetism (and that increases with particle size), is higher for the 100 Fe loading factor sample as compared to the 10 min sample, with the 60 min sample having an intermediate value. These data indicate that not all the iron in the 10 and 60 minute sample particle has completely mineralized to pure maghemite. It also indicates that the sample prepared by slower addition, and subsequent annealing, results in nearly complete maghemite formation.

In addition, when the suspension NMR dispersion profiles of the samples characterized in Figure 10 were investigated it was found that the T1 relaxivities dropped from 94-139 (100 Fe loading factor), 66-102 (60 minute sample), and 22-41 (10 min sample) at 2 MHz to 6-18 $mM^{-1}s^{-1}$ at 62 MHz (Fig. 11A). At the lowest frequencies, there was no decrease in 1/T1 for the 100 Fe loading factor sample, with a slight decrease but no full T1 relaxivity peak for the 60 minute sample. The 10-minute sample, however, exhibited a more pronounced peak of lower magnitude, which is expected for smaller, less magnetic materials according to recently developed theories for (super)paramagnetic nanoparticles. The magnitude of the 1/T1 at higher frequency is relatively low as compared to other magnetite-containing nanoparticles, likely as a result from a low surface to volume ratio (i.e. a larger particle oligomer). On the other hand, the measured T2 relaxivities (Fig. 11B) demonstrate a saturation at a relatively low frequency (between 10-20 MHz), with unusually high values of 240-400 and 250-380 $mM^{-1}s^{-1}$ for the 100 Fe loading factor sample and 60 minute sample, respectively. The high T2 values are indicative of the effectiveness of an MR contrast agent that includes the metal oxide-dendrimer composition. The magnitude of the 1/T2 data are typical of larger oligomeric structures, in which the individual magnetic crystals act together as one, larger particle. On the other hand, the T2 relaxivities of the 10 minute sample were significantly lower, between 63-125 mM^{-1} s^{-1}, in agreement with the smaller crystal size observed by TEM, chromatography, and magnetometry. There was

no interecho time dependence for 1/T2, indicating that the size regime of the mineralized dendrimers was well below the micrometer range. Thus, the magnetic behavior of this material is consistent with a small oligocrystalline assembly in suspension, and can be manipulated by varying the reaction conditions.

A reversible particle aggregation of the metal oxide-dendrimer composition suspensions could be induced by the application of a high magnetic field gradient. This was achieved through application of a small CoSm magnet to the suspension container, whereupon there was complete aggregation of all material. Upon removal of the magnetic field complete solubility was restored. This behavior is indicative of a soluble superparamagnetic material having no remnant magnetization at room temperature. In addition, these suspensions could be freeze-dried to powders that re-dissolved completely upon addition of water.

According to another example a mixture of divalent cobalt and iron ions (as NO_3^- or SO_4^{2-} salts) with ratios of $Co^{2+}_{1-x}Fe^{2+}_x$ in the range from x = 0 to 1, was added to an aqueous suspension of carboxyl-terminated PAMAM dendrimer at pH>8 and oxidized, by addition of a stoichiometric amount of oxidant ($(CH_3)_3)NO$, H_2O_2 or KIO_3), while under an inert atmosphere at temperatures of 60-65°C. When the reaction was complete, citrate was added to reach a final concentration between 1 and 10mM. The resulting minerals ranging in composition from Fe_3O_4, $FeCo_2O_4$ to Co_3O_4 were rendered soluble through their association with the carboxy-terminated dendrimer.

Example B – MR Contrast Agent

The metal oxide-dendrimer compositions used in Example B were made according to the process described above in Example A. In general, under anaerobic conditions, carboxy-terminated dendrimers (generation 4.5, commercially available from Dendritech) in 0.1M NaCl buffer, pH=8.5, were mixed with $(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$ at a loading factor of 100 iron atoms per dendrimer molecule.

The mixture was then oxidized with the 2 electron oxidant Me_3NO to yield a homogeneous brown suspension. The suspension pH was maintained by H^+ titration in a pH-Stat experiment (Brinkmann 718). Following termination of the reaction 35 mM sodium citrate was added to bind any non-bound or released Fe ions. The resulting aqueous suspensions (referred to herein as "MD-100") were further purified and concentrated by ultrafiltration (MW cutoff, 100 KDa) and extensive dialysis.

Further mineral analysis indicated a highly ordered inverse spinel maghemite- or magnetite-like structure, with a high saturation magnetization (M_{sat}) of 94 emu g/Fe, and no hysteresis at 300K. For comparison, the M_{sat} of pure magnetite (Fe_3O_4) and maghemite (γFe_2O_3) is 127.1 and 108.7 emu g/Fe, respectively; for MION-46L (a dextran-coated magnetic nanoparticle), the magnetization at 1.5 Tesla is 60-68 emu g/Fe.

Cells were magnetically labeled by simply adding MD-100 to a culture medium at concentrations of up to 25 μg Fe/ml and incubation periods of 1-2 days. Prussian Blue staining of magnetically tagged cells showed a remarkable high degree of intracellular labeling, with the cytoplasm containing large numbers of iron-containing vesicles or endosomes (see Fig. 12; E and F show a comparison between non-enhanced and diamino-benzidine (DAB)-enhanced Prussian Blue stains with the latter being more sensitive to detect iron). Achievement of intracellular labeling is a critical requirement, since a membrane-bound magnetic probe is likely to interfere with cell-tissue interactions (membrane recognition processes), may detach easily from the cell membrane, or may be taken up and transferred to other cells *in vivo*.

Human HeLa and GLC-28 cells, rat CG-4 cells, and mouse 3T3 and C2C12 cells all showed a comparable degree of uptake (Fig. 12), demonstrating that the MD-100 uptake is non-specific and not dependent on the cell type or species. All cells remained fully viable with no difference in growth characteristics between labeled and unlabeled cells. Magnetically labeled rat CG-4 and human HeLa cells were further analyzed by relaxometry. The 1/T1 of labeled cell suspensions showed a significant increase as compared to non-labeled controls for the higher cell concentrations tested

(Fig. 13A). Under the same conditions, the 1/T2 increased dramatically from approx. 1 to 50 (CG-4) and 150 (HeLa) s^{-1} (Fig. 13B).

Fig. 13C shows the corresponding iron contents of the cell samples measured using 2 different methods. There is a good agreement between the amount of iron and increase in relaxation rates. Using the relaxometric Fe assay, the cellular uptake was calculated to be 18.6 ± 8.5 and 27.1 ± 11 pg Fe/cell for the CG-4 and HeLa cells, respectively. The corresponding values obtained using the Ferrozin assay were 17.0 ± 4.0 and 27.2 ± 5.8, respectively. The "cellular relaxivity" (i.e. increase in cellular relaxation rates per unit Fe concentration) was 0.90-1.01 (CG-4) and 1.10-1.31 $s^{-1}mM^{-1}$ Fe (HeLa) for 1/T1, calculated for 0.1 Tesla using the two different iron assays. At the same field strength, the cellular T2 relaxivity was calculated to be 23.5-30.0 (CG-4) and 33.2-38.9 $s^{-1}mM^{-1}$ Fe (HeLa). These values indicate a near 100-fold reduction in T1 relaxivity for MD-100 when going from an unbound, free-in-suspension state to a state representing intracellular compartmentalization in endosomes. On the other hand, the cellular T2 relaxivity decreases only about 10-fold because there can be a significant remaining outer-sphere relaxation effect for dipole-dipole or spin-spin type interactions that does not contribute to 1/T1, in addition to dephasing and T2* susceptibility effects (which were kept small by the short interecho time of refocusing 180° pulses that was used in the CPMG experiments). The overall result is a net increase in the 1/T2 to 1/T1 ratio which translates to an increase in the relative hypointensity of MR contrast when using standard imaging sequences.

Following magnetic labeling, HeLa cells in suspension could be rapidly (i.e., within 5-10 seconds) attracted and separated (see Figs. 14A-14D) using a 1200 Gauss/cm permanent NdFeB magnet (Fig. 14F). Following removal of the magnet, the cells could be fully re-suspended and did not clump together. Such a characteristic could provide for magnetic manipulation of cells (e.g., enrichment of weakly vs. strongly labeled cells) before transplantation.

It was also found that human multipotent neural stem cells (NSCs) could be highly labeled at a MD-100 incubation concentration of only 2.5 μg Fe/ml (see Fig. 15). Magnetically labeled human NSCs exhibited a similar growth rate as unlabeled cells, and showed a comparable formation of neuronal processes when replated and grown for an additional 10 days. Morphologically, labeled cells resembled differentiation into glial cells and neurons, and the latter was further demonstrated by immunospecific staining using the MAP-2 monoclonal antibody (see Fig. 15D). In a separate experiment, rat NSCs were allowed to first differentiate into oligodendroglial progenitors, and then labeled with MD-100 for 24h at 25.0 μg Fe/ml. These cells also showed a considerable intracellular uptake of MD-100 (Fig. 15E), demonstrating that stem cells may be labeled either before or after differentiation into the desired cell type.

We also assessed MD-100 labeling of human multipotent mesenchymal stem cells (MSCs). MD-100 labeled MSC (Fig. 15F) showed a dose-dependent increase in the number and density of iron-containing vesicles and relaxation rate enhancement, with both assessments demonstrating a significant labeling at even the lowest MD-100 tested (1 μg Fe/ml). Effective MD-100 labeling of MSC at different dosages is further demonstrated in Figures 16B-16D. When MSC were re-plated at approximately 10-20% confluence and re-cultured for an additional 6 days, they quickly proliferated to full confluence at a similar rate as unlabeled cells. The 1/T2 relaxation rate and Fe content of re-cultured cells remained significantly elevated at about 50 % of the corresponding values before re-plating. These results demonstrate a considerable probe longevity and a prolonged storage of MD-100 within endosomes.

A further experiment involved detecting MD-100 labeled cells *in vivo*. Rat NSCs were differentiated into oligodendroglial progenitors, transfected with the LacZ gene, labeled with MD-100, and injected into both lateral ventricles of neonatal (P0) Long Evans shaker (*les*) rats. These animals are dysmyelinated mutants resulting from a defect in the gene encoding for myelin basic protein. Animals were imaged at weekly to biweekly intervals to determine the tissue distribution of transplanted cells. Figure 17A shows an MR image obtained *in vivo* using a 4.7 Tesla animal MR imaging unit 6

weeks post transplantation. Migration of labeled cells into the brain parenchyma can be clearly detected, with a good anatomical correlation with the macroscopic distribution of β-galactosidase-expressing cells (Fig. 17B). Note that the area of MR contrast appears to spread somewhat further into the brain parenchyma; this is partially related to the fact that we used a T2*-sensitive 3D imaging sequence that is very sensitive to MD-100-induced local disturbances in the magnetic field. This causes water protons at more remote sites to be affected as well, leading to a "blooming effect", i.e. an amplification of signal changes.

Another experiment demonstrated *in vivo* tracking of tumor growth. The human small cell lung carcinoma (SCLC) cell line GLC-28 (5) was labeled with 10 μg Fe/ml MD-100 for 48 hours. Approximately 5 x 10^6 cells were implanted in the flank of 6 weeks old nude rats, along with unlabeled cells as control. Tumor growth was followed serially *in vivo* on a weekly basis at 234x 375 μm resolution (2 mm slices) using a clinical 1.5 T system (GE Signa) and a 3" surface coil (see Figs. 18A and 18B) and *ex vivo* at 100 or 50 μm isotropic resolution using a 4.7 T GE CSI Omega NMR spectrometer, a 3D multi-gradient echo and a 15 mm diameter home-made surface coil (see Figs. 18C and 18D). Imaged tumor specimens were processed for routine tissue histology including iron-specific (DAB-enhanced) Prussian Blue stains (Fig. 18E). Implanted magnetically labeled and unlabeled tumor cells exhibited similar growth rates, indicating that MD-100 can be considered a "vital dye" (i.e., a compound that can mark cells without damaging them). At the earlier timepoints (tumor size approx. 5-10 mm) the labeled neoplasms appeared uniformly black on the images. At later timepoints (tumor size approx. 10-30 mm) unusual cellular migration patterns were seen, mainly from the dark center protruding towards the outer rim, with distinct clusters of hypointense labeled cells. The outer rim of the tumor usually lacked hypointense cells and was isointense to the untagged control tumors, as a result from multiple cell divisions and associated MD-100 dilution effects. Little or no necrosis was observed, and control tumors did not exhibit any hypointensity due to hemorrhage. Figures 18C and 18D show high-resolution *ex vivo* tumor images demonstrating an

organized contrast pattern with multiple single pixel spreads. Figure 18E shows the correlating DAB-enhanced Prussian Blue staining for the image in Figure 18D. It can be appreciated that the iron staining matches the area of MR contrast, with each pixel corresponding to only a few cells. Given that the MR voxel size dimension is about 5-10 times greater (in each dimension) than the diameter of a single cell, outer sphere magnetic susceptibility effects can thus significantly enhance cellular contrast, that is, extend far beyond the surface of the cell.

Cell culture and magnetic labeling - experimental protocol for Example B

HeLa human cervix carcinoma, GLC-28 human small cell lung carcinoma, CG-4 rat oligodendrocyte progenitor, NIH 3T3 mouse fibroblast, and C2C12 mouse muscle progenitor cells were maintained as permanent cell lines. Human NSCs were isolated from the ventral midbrain of anonymous post-mortem human fetal donations (provided by NeuralStem Biopharmaceuticals). Human MSCs were derived from bone marrow of normal donors (BioWhittaker). Rat NSCs were isolated from the subependymal striatum of *les* rats . CG-4 cells, NSCs, and MSCs were maintained and labeled as undifferentiated cell lines (using appropriate growth factors). Rat NSC were differentiated into oligodendroglial progenitors (oligospheres) by growth factor treatment before magnetic labeling. All cells were co-cultured for 24-48 h in standard media containing MD-100 at a dose ranging from 1 to 25 μg Fe/ml. Cells were trypsinized (except GLC-28), washed three times, and then re-plated on Permanox® chamber slides or glass coverslips for an additional 24h or 6 (MSCs) and 10 (NSCs) days. NSCs were allowed to differentiate into neurons and glial cells by withholding growth factors.

Analysis of cellular magnetic labeling - experimental protocol for Example B

Cells were trypsinized and washed three times for further analysis. For Prussian Blue staining, cells were fixed with 4% glutaraldehyde, washed, incubated for 30 min with 2% potassium ferrocyanide (Perls' reagent) in 6% HCl, washed, and counterstained with nuclear fast red. For DAB-enhanced Prussian Blue staining, slides were not counterstained but instead reacted with un-activated and activated (containing 0.03% H_2O_2) 14% DAB for 15 min each. For immunoperoxidase-staining of NSCs, chamber slides were fixed with 4% paraformaldehyde, washed, incubated with mouse-anti rat MAP-2 monoclonal antibody for 45 minutes at room temp, washed, incubated with rabbit-anti-mouse Ig-HRP for 45 minutes at room temp, washed, incubated with 0.2 mg/ml solubilized 3-amino-9-ethyl-carbazole in 0.05 M acetate buffer, pH = 4.9 + 0.03% H_2O_2 for 10 minutes, washed, and counterstained with Mayer's haematoxylin.

For relaxometry of labeled cell suspensions, cells were counted using a hemocytometer, adjusted for cell concentration, and re-suspended in 500 µl 4% w/w gelatin. Using a variable field relaxometer (see above), the T1 and T2 were measured at room temp at 0.1 and 1.0 Tesla as described, and using a T2 interecho time of 2, 4, 6, and 10 msec. For assessment of the corresponding total iron content, the cell suspensions were first dried at 110 °C for 16 hours, and then completely digested in acid. To each sample, 375 µl 70 % ultrapure perchloric acid (containing < 2 ppb Fe) , and 125 µl 100 % ultrapure nitric acid (< 0.6 ppb Fe) was added, and samples were digested for 3 hours at 60 °C using a heating block. For these 500 µl samples, the 1/T1 and 1/T2 were then measured at room temp and at 0.1 and 1 Tesla as described above. Calibration standards of ferrous chloride containing 0.2-10.0 mM Fe in the same acid mixture were included. The relationship between the relaxation rates and Fe concentration was linear for the entire Fe range measured, with slopes of 10.2-13.4 s^{-1}/mM Fe for 1/T1 ($r > 0.99$) and 11.7-13.3 s^{-1}/mM Fe for 1/T2 ($r > 0.99$). The iron content of the cell samples was calculated for 1/T1 and 1/T2 for both field strengths, and the average Fe concentration was determined from these 4 sets of data. The iron content was then further determined and validated by a Ferrozine-based

spectrophotometric assay , using triplicate 50 µl samples from the acid-digested cell suspensions. The iron content per cell was calculated for each cell concentration and expressed as the average ± SD.

For MR imaging of labeled MSC suspensions, washed cells were counted using a hemocytometer, and re-suspended in 100 µl 1% low-melting agarose at a cell density of 1 x 10^7 cells/ml. The gel suspension was then transferred into a 24 well plate that was preloaded with agarose. The plate was imaged at 1.5 T (Signa, General Electric) using a 3" surface coil and several different clinical pulse sequences. Images were obtained with a FOV of 6x6 cm and a 1.5 mm slice thickness.

In vivo tracking of magnetically labeled cells - experimental protocol for Example B

Rat oligodendroglial progenitors (oligospheres) were prepared from NSC as described above, transfected with the LacZ reporter gene, triturated, and labeled with MD-100 for 24h at 25 µg Fe/ml. Cells were washed, and approximately 5 x 10^4 cells in 2-3 µl medium were transplanted into both lateral intracerebroventricular regions of neonatal (P0) *les* rats (n=5). These experiments were conducted in accordance with institutional guidelines for the use and care of laboratory animals. Under general isoflurane anesthesia, pups were imaged at 1.5 T (General Electric, Signa) at 18, 25, 31, and 44 days post grafting, using a quadrature wrist coil and several different clinical pulse sequences. 2D images were obtained at 2 mm slice thickness; a 1.2 mm thick 3D SPGR data set was also obtained. In addition, at 21 and 42 days post grafting, 3D multi-gradient echo MR images were obtained at 313 µm isotropic resolution using a 4.7 T GE CSI Omega NMR spectrometer and a 1.5 inch diameter home-made surface coil. The latter parameters were: FOV = 4 x 3 x 3 cm; matrix = 128 x 96 x 96; NEX = 1; TR = 100 msec; TE = 6 msec; n echoes = 6, flip angle = 15 deg. Following imaging, paraformaldehyde-perfused brain specimens were cut into 2 mm slices for gross morphology and stained with X-gal for β-galactosidase (LacZ) expression.

Having illustrated and described the principles of our invention with reference to several embodiments, it should be apparent to those of ordinary skill in the art that the invention may be modified in arrangement and detail without departing from such principles.

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We claim:

1. A magnetic probe comprising an aqueous suspension that includes magnetic-responsive metal (oxyhydr)oxide particles stabilized by a dendrimer.

2. A probe according to claim 1 wherein the metal (oxyhydr)oxide particles comprise metal oxide particles.

3. A probe according to claim 2 wherein the metal oxide particles comprise superparamagnetic iron oxide particles.

4. A magnetic probe comprising an aqueous suspension that includes metal (oxyhydr)oxide particles formed in the presence of a dendrimer.

5. A probe according to claim 4 wherein the metal atom in the metal (oxyhydr)oxide is selected from iron, cobalt, gadolinium, europium or manganese.

6. A probe according to claim 4 wherein the dendrimer is selected from poly(amidoamine) or poly(propyleneimine).

7. A magnetic resonance contrast agent comprising an aqueous suspension that includes iron oxide particles synthesized in the presence of a carboxyl-terminated poly(amidoamine) dendrimer.

8. A magnetic resonance contrast agent comprising an iron oxide-dendrimer complex.

9. A contrast agent according to claim 8 wherein the complex is substantially free of a metal chelating agent.

10. A magnetic resonance analysis contrast agent comprising an aqueous suspension produced by:

contacting a ferrous or ferric ion-containing material with a dendrimer; and

oxidizing the ferrous or ferric ion-containing material in the presence of the dendrimer to form an iron oxide.

11. A contrast agent according to claim 10 wherein the dendrimer comprises a carboxy-terminated dendrimer.

12. A method of labeling living cells to render the cells magnetic resonance sensitive comprising:

contacting a metal (oxyhydr)oxide-dendrimer composition with the cells to be labeled; and

allowing the metal (oxyhydr)oxide-dendrimer composition to be internalized by the cell.

13. A method according to claim 12 wherein the metal atom in the metal (oxyhydr)oxide is selected from iron, cobalt, gadolinium, europium or manganese.

14. A method according to claim 12 wherein the metal (oxyhydr)oxide-dendrimer composition comprises an aqueous suspension that includes iron oxide particles formed in the presence of the dendrimer.

15. A method according to claim 12 wherein the metal (oxyhydr)oxide-dendrimer composition comprises an iron oxide-dendrimer complex.

16. A method according to claim 12 wherein the cells are selected from stem cells and neural cells.

17. A method according to claim 12 wherein the metal (oxyhydr)oxide-dendrimer composition enters into the cellular cytoplasm.

18. A method according to claim 12 wherein the contacting step comprises culturing the cell to be labeled in a medium that includes the metal (oxyhydr)oxide-dendrimer composition.

19. A method of labeling living cells to render the cells magnetic resonance sensitive comprising:

contacting a metal oxide-dendrimer composition with the cells to be labeled; and

allowing the metal oxide-dendrimer composition to be internalized by the cell.

20. A method of detecting living cells in a host comprising:

contacting a metal (oxyhydr)oxide-dendrimer composition with the cells;

allowing the metal (oxyhydr)oxide-dendrimer composition to be internalized by the cells; and

imaging the host with magnetic resonance imaging so as to detect the cells.

21. A method according to claim 20 wherein the metal atom in the metal (oxyhydr)oxide is selected from iron, cobalt, gadolinium, europium or manganese.

22. A method according to claim 20 wherein the metal (oxyhydr)oxide-dendrimer composition comprises an aqueous suspension that includes iron oxide particles formed in the presence of the dendrimer.

23. A method according to claim 20 wherein the metal (oxyhydr)oxide-dendrimer composition comprises an iron oxide-dendrimer complex.

24. A method according to claim 20 wherein the metal (oxyhydr)oxide-dendrimer composition is a magnetic resonance imaging contrast agent.

25. A method according to claim 20 wherein the cells are selected from stem cells and neural cells.

26. A method according to claim 20 wherein the metal (oxyhydr)oxide-dendrimer composition enters into the cellular cytoplasm.

27. A method according to claim 20 wherein the contacting step comprises culturing the cell to be labeled in a medium that includes the metal (oxyhydr)oxide-dendrimer composition.

28. A method of manipulating cells comprising:

contacting a magnetic-responsive metal (oxyhydr)oxide-dendrimer composition with the cells;

allowing the magnetic-responsive metal (oxyhydr)oxide-dendrimer composition to be internalized by the cells;

providing a suspension that includes the magnetically-tagged cells; and

subjecting the magnetically-tagged cells to a magnetic field to attract the magnetically-tagged cells.

29. A method according to claim 28 further comprising removing the magnetic field so as to re-suspend the magnetically-tagged cells.

30. A composition comprising an iron oxide-carboxylated dendrimer complex.

31. A composition according to claim 30 wherein the complex is substantially free of a metal chelating agent.

32. A composition according to claim 30 wherein the carboxylated dendrimer comprises a carboxy-terminated poly(amidoamine) dendrimer.

33. A composition comprising an iron oxide-dendrimer complex wherein the iron oxide is bound to a dendrimer molecule at an outer external surface of the dendrimer molecule.

34. A water soluble aggregate comprising:

a metal (oxyhydr)oxide domain that includes more than one discrete particle;

a dendrimer domain that includes more than one generally spherical dendrimer molecule, each molecule having a peripheral external surface;

wherein the metal (oxyhydr)oxide domain is substantially confined within the dendrimer domain by the peripheral external surfaces of the dendrimer molecules.

35. An aggregate according to claim 34 wherein the metal (oxyhydr)oxide domain comprises a crystalline structure.

36. An aggregate according to claim 34 wherein the crystalline structure comprises at least one of magnetite or maghemite.

37. An aggregate according to claim 34 wherein the aggregate has a diameter of at least about 15 nm.

38. An aqueous suspension comprising metal (oxyhydr)oxide particles stabilized by a dendrimer.

39. An aqueous suspension according to claim 38 wherein the metal (oxyhydr)oxide particles are magnetic-responsive iron oxide particles.

40. An aqueous suspension according to claim 38 wherein the metal (oxyhydr)oxide particles are metal oxide particles.

41. An aqueous suspension according to claim 40 wherein the metal oxide particles are superparamagnetic particles.

42. An aqueous suspension comprising metal (oxyhydr)oxide particles formed in the presence of a dendrimer.

43. An aqueous suspension according to claim 42 wherein the metal atom in the metal (oxyhydr)oxide is selected from iron, cobalt, gadolinium, europium or manganese.

44. An aqueous suspension according to claim 42 wherein the dendrimer is selected from poly(amidoamine) or poly(propyleneimine).

45. An aqueous mixture of ingredients comprising:

(a) a ferrous or ferric ion-containing material; and

(b) a dendrimer having terminal groups selected from carboxy, sulfono, sulfonato, phosphono and phosphonato.

46. An aqueous mixture according to claim 45 further comprising an oxidizing agent.

47. An aqueous suspension comprising:

(a) iron oxide particles; and

(b) dendrimer molecules having terminal groups selected from carboxy, sulfono, sulfonato, phosphono and phosphonato.

48. A method of making a metal (oxyhydr)oxide-dendrimer composition comprising:
contacting a metal ion-containing material with a dendrimer having terminal groups selected from carboxy, sulfono, sulfonato, phosphono and phosphonato; and
oxidizing or hydrolyzing the metal ion-containing material in the presence of the dendrimer.

49. A method of making a metal oxide-dendrimer composition comprising:
contacting a metal ion-containing material with a dendrimer having terminal groups selected from carboxy, sulfono, sulfonato, phosphono and phosphonato; and
oxidizing the metal ion-containing material in the presence of the dendrimer.

50. A method according to claim 49 wherein the dendrimer comprises a carboxy-terminated poly(amidoamine) dendrimer.

51. A method according to claim 49 wherein the oxidizing comprises adding an oxidizing agent.

52. A method according to claim 51 wherein the oxidizing agent is selected from $(CH_3)_3NO$, H_2O_2, KIO_3, or O_2.

53. A method according to claim 49 further comprising adding a metal chelating agent to remove the non-oxidized metal ion-containing material.

54. A method of making an iron oxide-dendrimer composition comprising:
contacting a ferrous or ferric ion-containing material with a dendrimer; and

oxidizing the ferrous or ferric ion-containing material in the presence of the dendrimer to form an iron oxide.

55. A method according to claim 54 wherein the oxidizing comprises adding an oxidizing agent selected from $(CH_3)_3NO$, H_2O_2, KIO_3, or O_2.

56. A method of making an aqueous suspension that includes superparamagnetic iron oxide particles comprising:

mixing a ferrous or ferric ion-containing material with a carboxy-terminated dendrimer; and

oxidizing the ferrous or ferric ion-containing material in the presence of the carboxy-terminated dendrimer to form suspended superparamagnetic iron oxide particles.

57. A method of making an aqueous suspension that includes superparamagnetic iron oxide particles comprising mixing together in an aqueous medium the following ingredients:

(a) a carboxy-terminated dendrimer;

(b) a material that includes at least one of Fe^{2+} or Fe^{3+} ions; and

(c) an oxidizing agent.

58. A method for synthesizing metal oxide nanoparticles comprising *in situ* oxidation of a metal ion-containing material in the presence of a dendrimer.

59. A method of making an iron oxide-dendrimer complex comprising:

(a) adding at least one of Fe^{2+} or Fe^{3+} ions to an aqueous suspension of carboxy-terminated poly(amidoamine) dendrimer to form an intermediate complex; and

(b) oxidizing the intermediate complex.

60. A method according to claim 59 wherein step (a) comprises adding at least one of $(NH_4)_2Fe(SO_4)$, $FeCl_2$ or $FeSO_4$ to the dendrimer suspension.

61. A method according to claim 59 wherein the oxidizing further comprises heating the intermediate complex/oxidizing agent mixture at a temperature of at least about 50°C.

62. A method according to claim 59 further comprising adding a metal chelating agent after the oxidizing step to remove any non-oxidized metal ion-containing material.

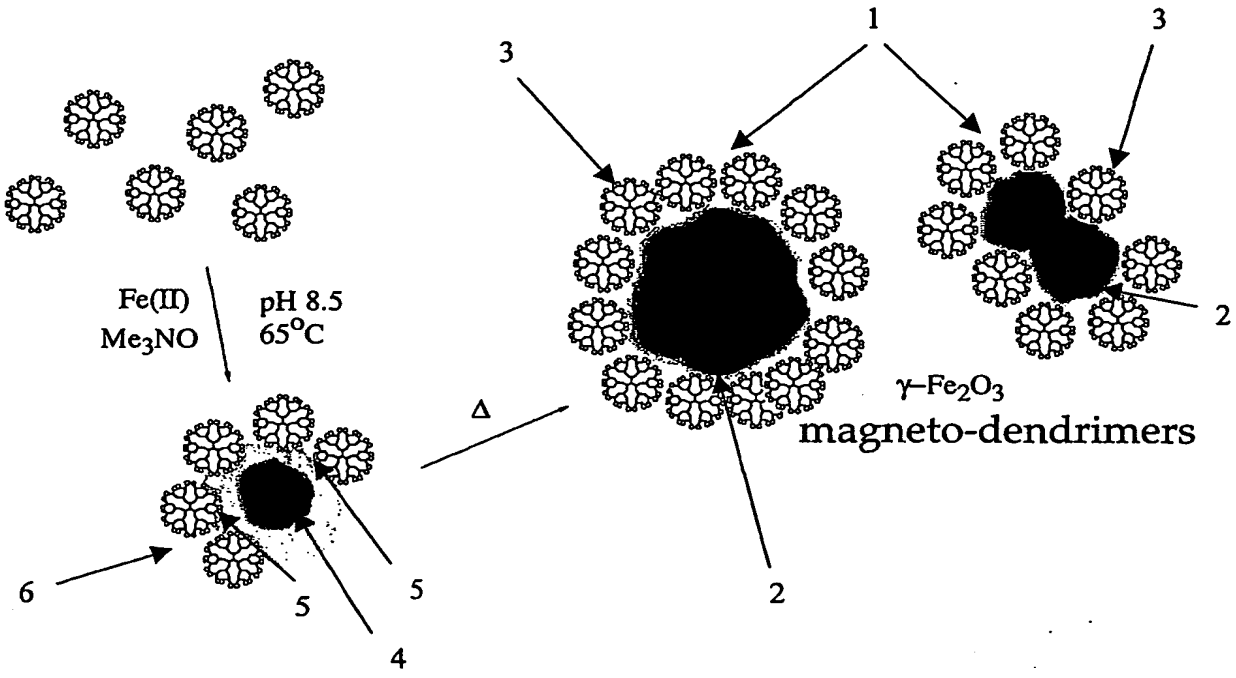


FIG. 1

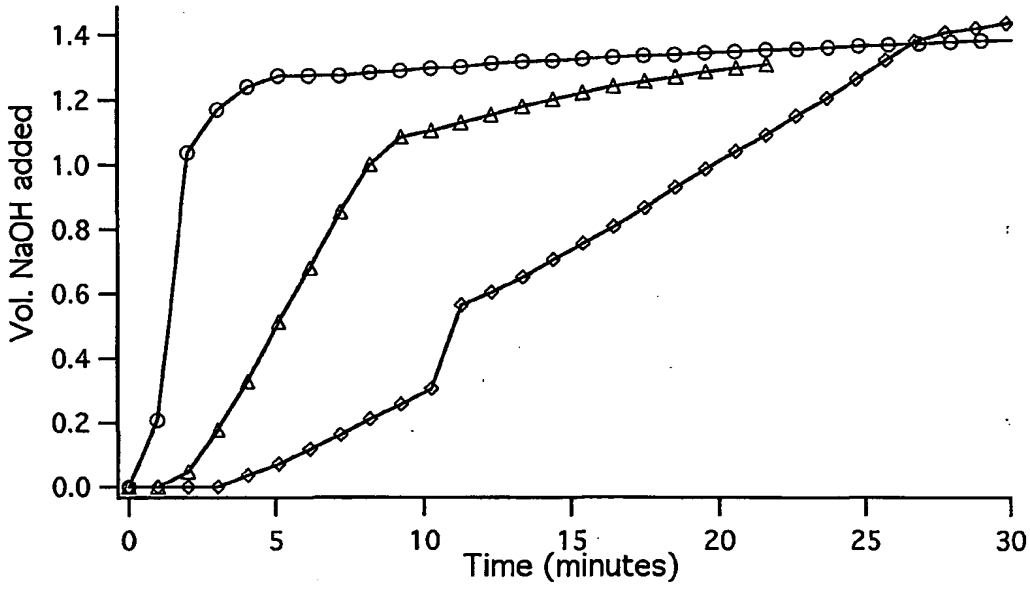

Vol. NaOH added
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
5
10
15
20
25
30
Time (minutes)

FIG. 2

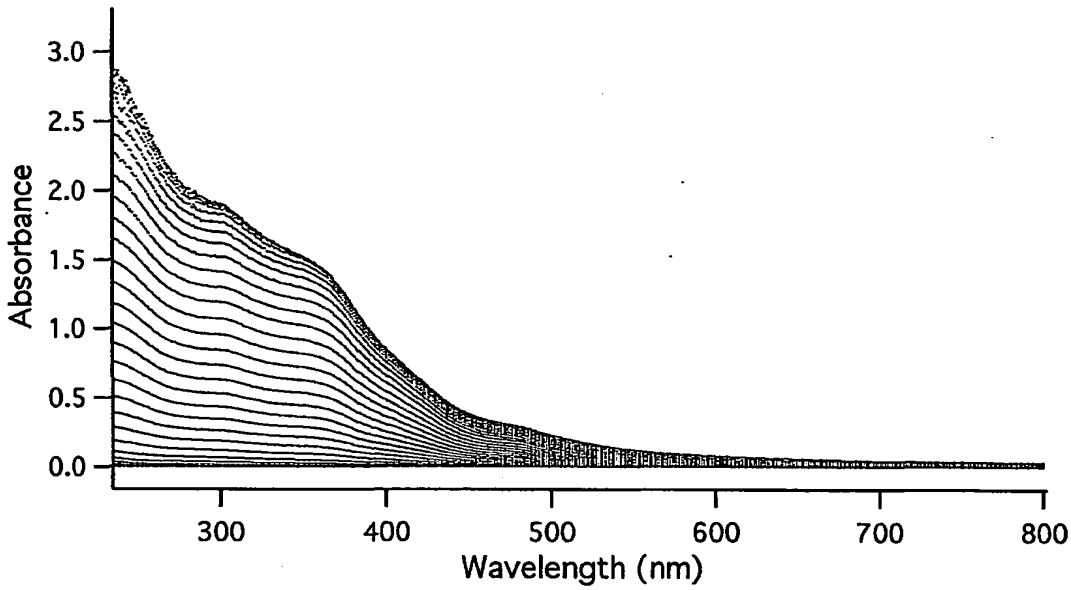

3.0
2.5
2.0
1.5
1.0
0.5
0.0
Absorbance
300
400
500
600
700
800
Wavelength (nm)

FIG. 3

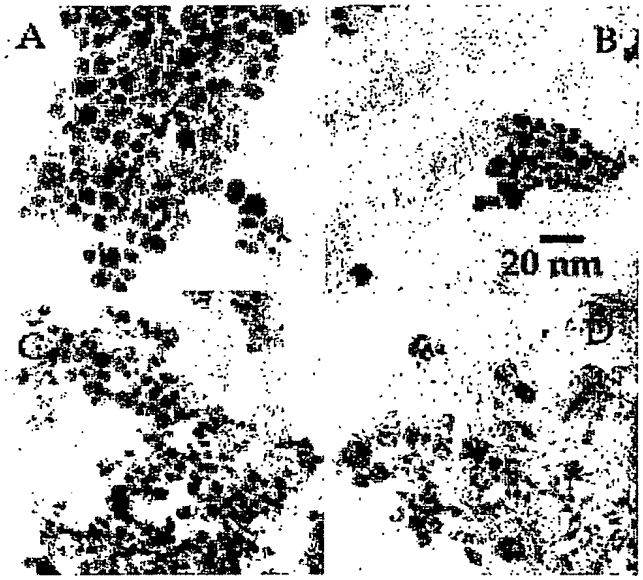


FIG. 4

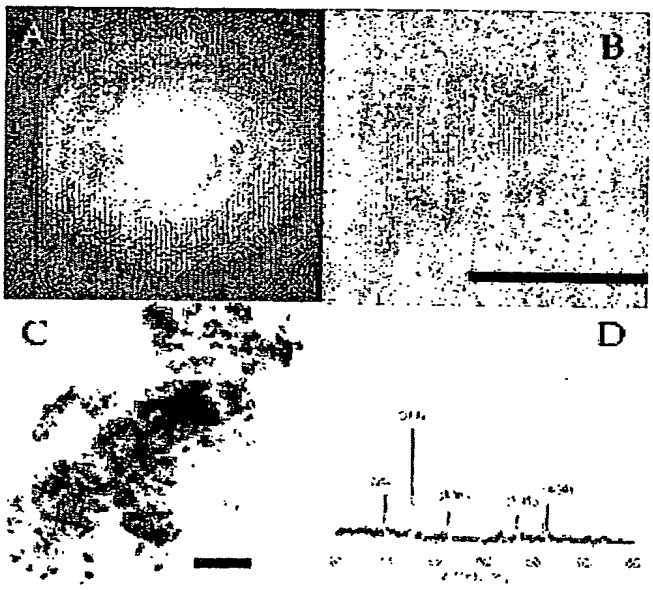


FIG. 5

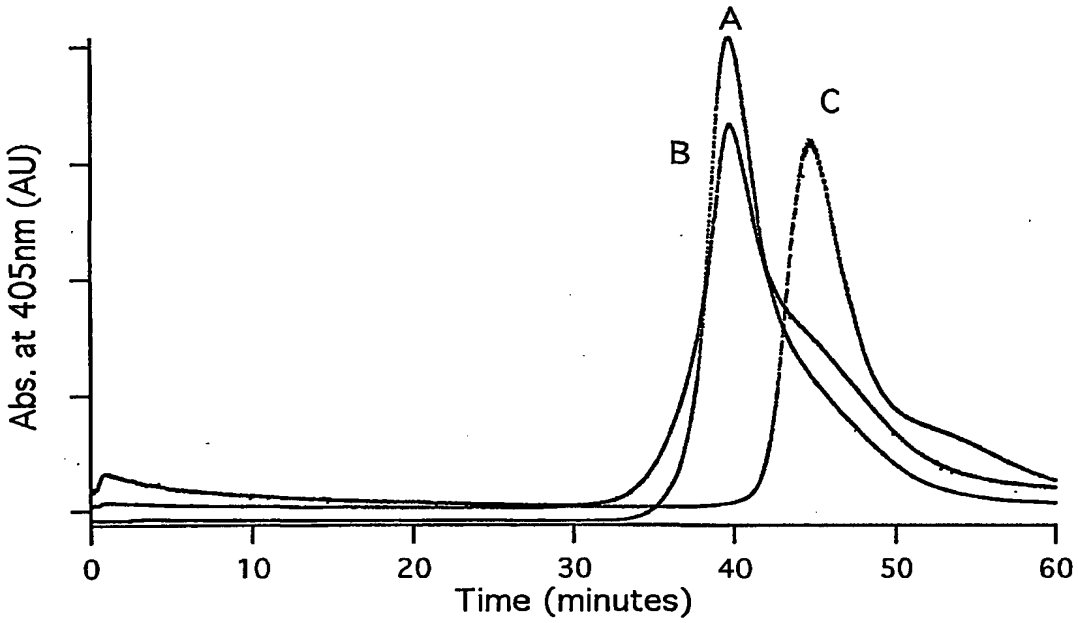


FIG. 6

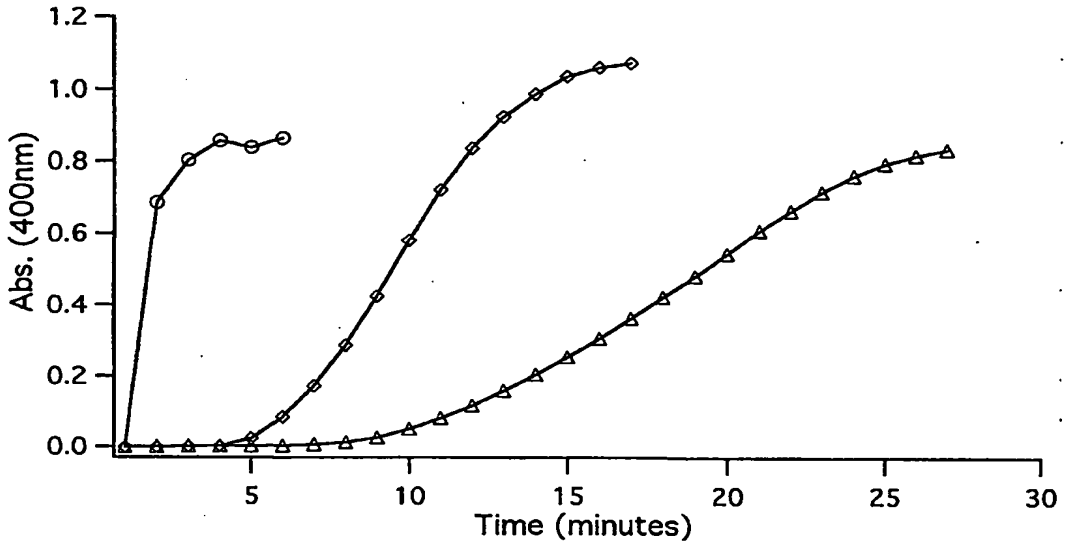

1.2
1.0
0.8
0.6
0.4
0.2
0.0
Abs. (400nm)
5
10
15
20
25
30
Time (minutes)

FIG. 7A

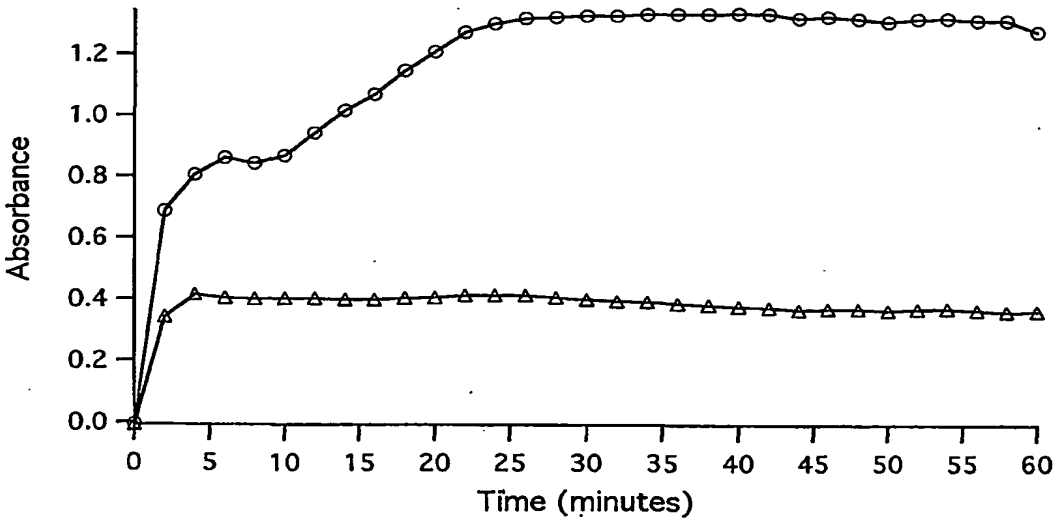

1.2
1.0
0.8
0.6
0.4
0.2
0.0
Absorbance
0
5
10
15
20
25
30
35
40
45
50
55
60
Time (minutes)

FIG. 7B

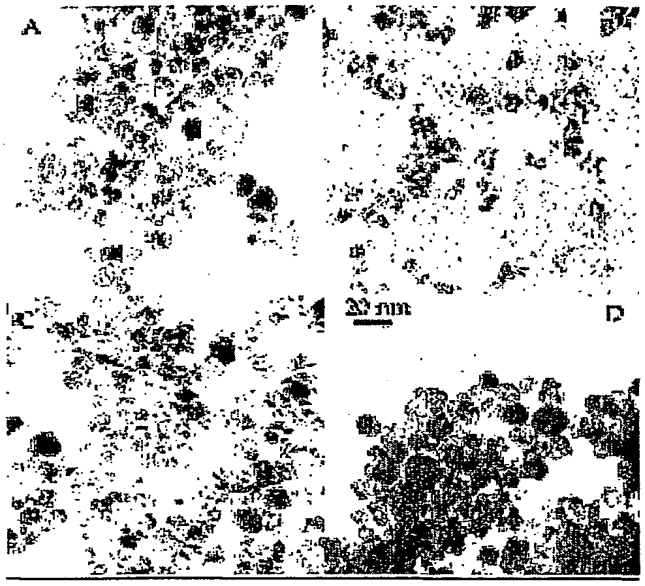


FIG. 8

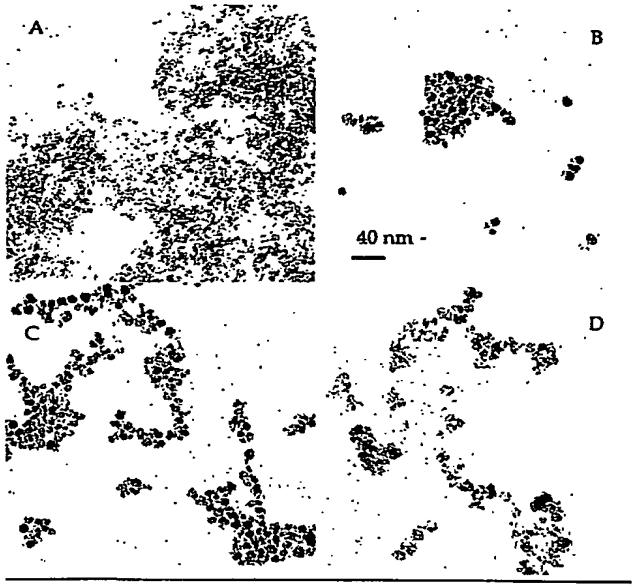


FIG. 9

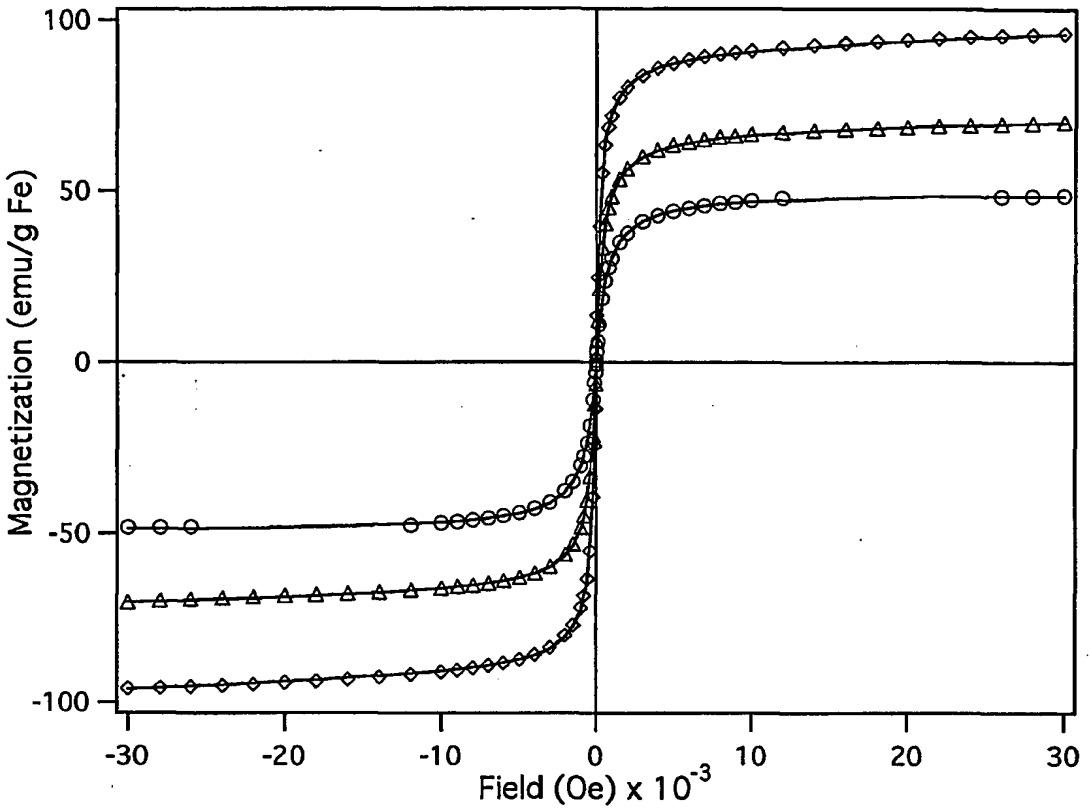

100
50
0
-50
-100
Magnetization (emu/g Fe)
-30
-20
-10
0
10
20
30
Field (Oe) x 10^-3

FIG. 10

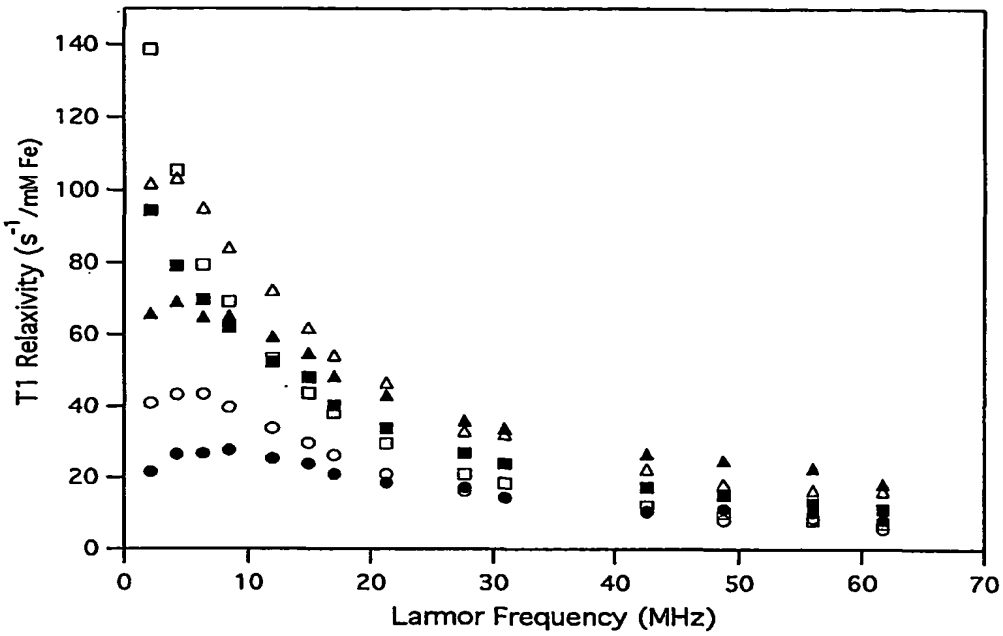


FIG. 11A

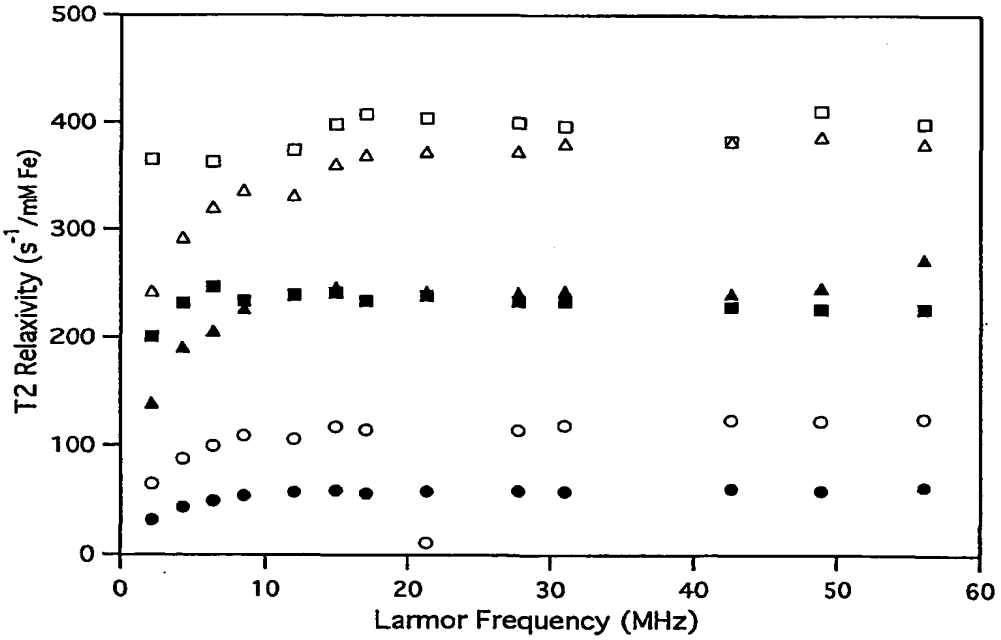


FIG. 11B

FIG. 12A

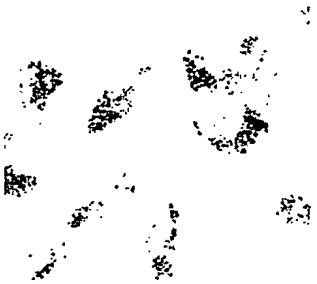


FIG. 12B

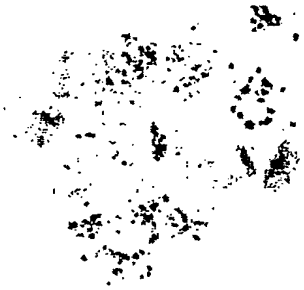


FIG 12C

FIG. 12D

FIG. 12E

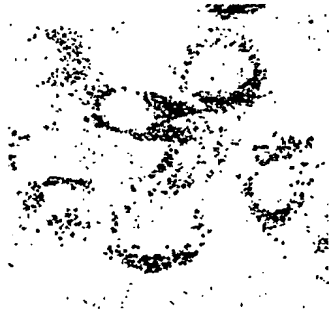


FIG. 12F

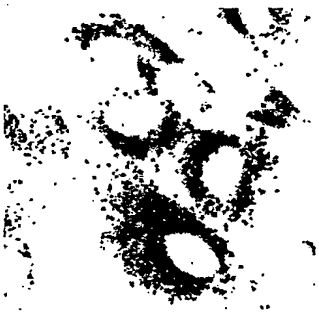


FIG. 13A

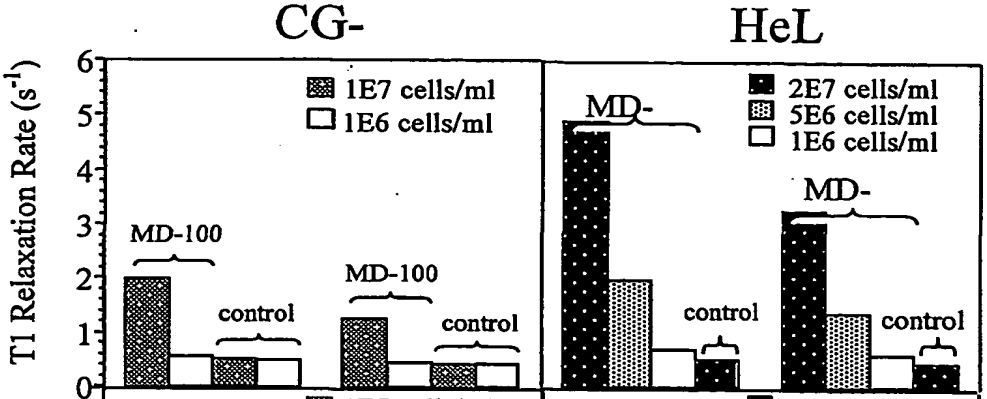


FIG. 13B

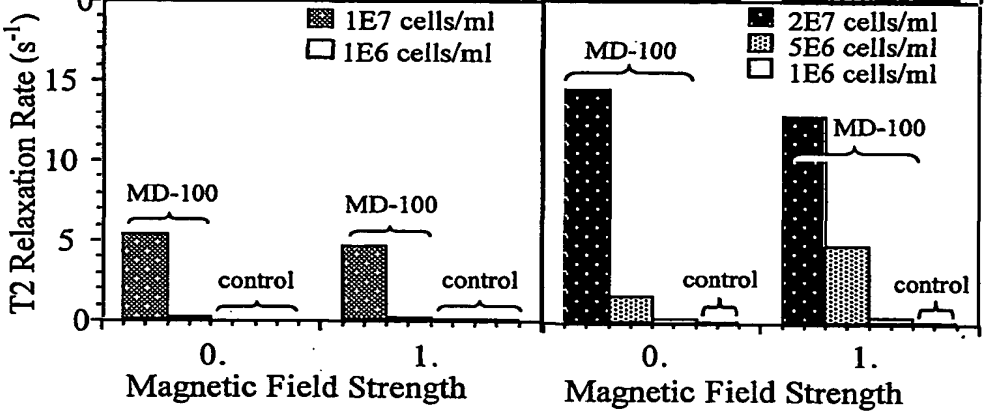
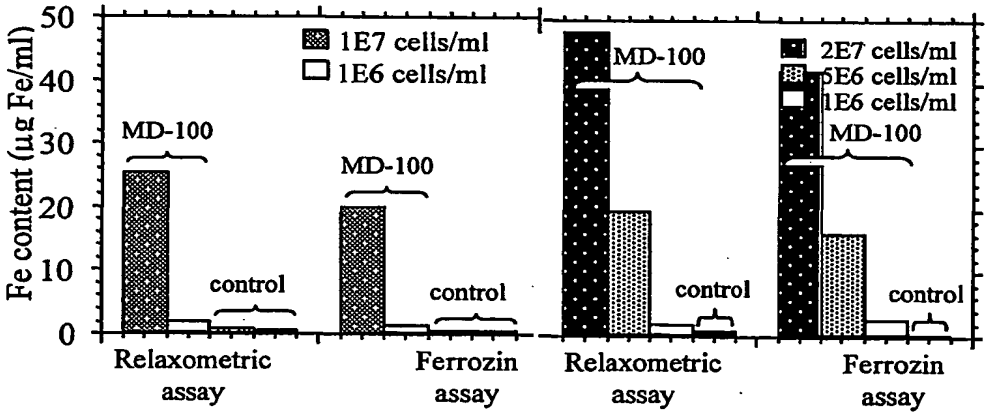


FIG. 13C



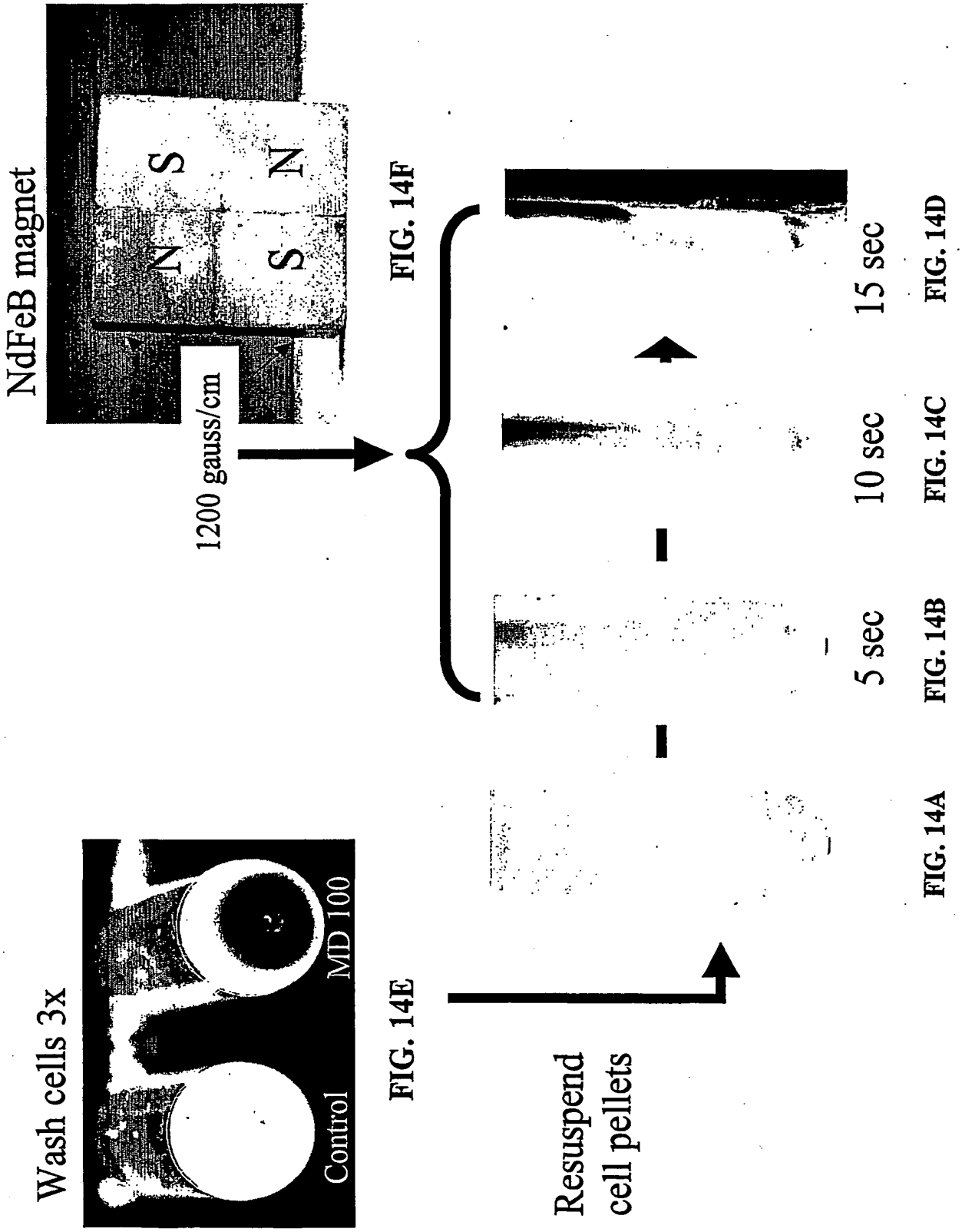


FIG. 14A FIG. 14B FIG. 14C FIG. 14D FIG. 14E FIG. 14F

FIG. 15A

FIG. 15B

FIG. 15C

FIG. 15D

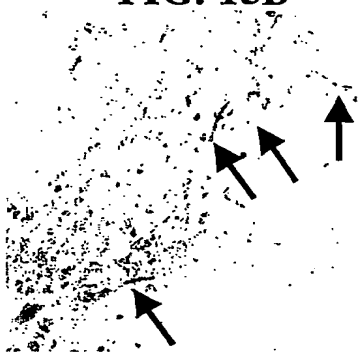


FIG. 15E

FIG. 15F

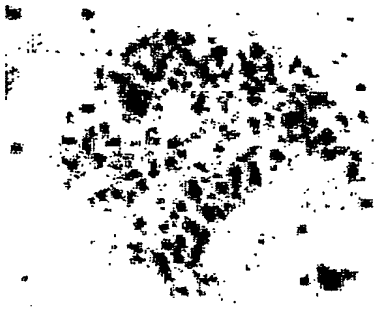


FIG. 16A FIG. 16B FIG. 16C FIG. 16D

FIG. 17A

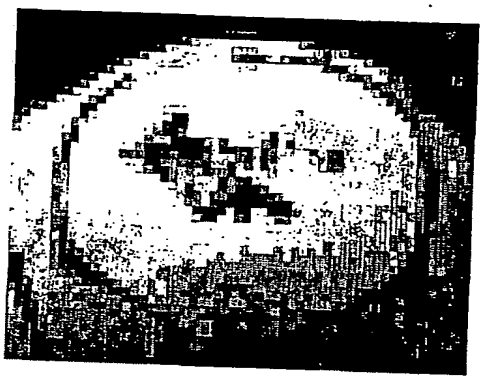
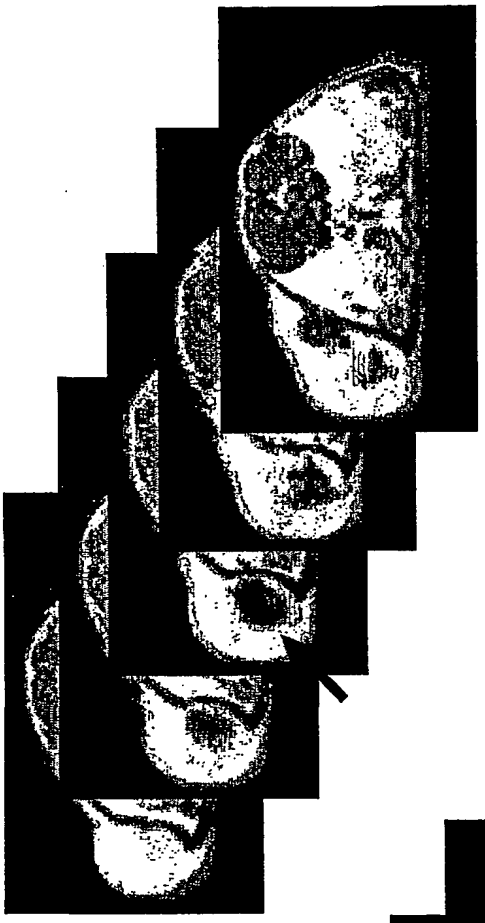


FIG. 17B



27 days following implantation

FIG. 18B

20 days following implantation

FIG. 18A

FIG. 18C

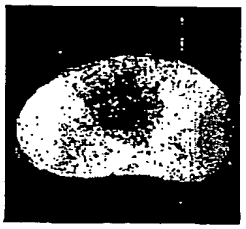


FIG. 18D

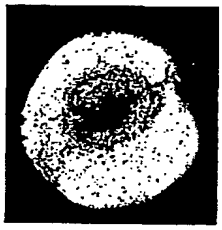


FIG. 18E